SYNTHETIC PORPHYRINS AS OXYGEN-CARRIERS

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DEGREE OF DOCTOR OF PHILOSOPHY

by

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of

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TO MY PARENTS

PREFACE

This dissertation is the result of work of the author carried out at the University Chemical Laboratory, Lensfield Road, Cambridge, between October 1974 and September 1977. It includes nothing which is the outcome of work done in collaboration. It has not been submitted in whole or in part for a degree or other qualification at another university.

In accordance with the regulations of the Degree Committee of the Faculty of Physics and Chemistry, this dissertation does not exceed 60 000 words in length.

M. D. Turnbull.

ACKNOWLEDGEMENTS

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I acknowledge financial support from the Science Research Council, in the form of an "Assist" Award.

ABBREVIATIONS

b.p. : Boiling point

m.p. : Melting point

n.m.r. : Nuclear magnetic resonance

t.f.a. : Trifluoroacetic acid

T.M.S. : Tetramethylsilane

a haem : Any iron-containing porphyrin

-Ac : $-COCH_3$

$-^tBu$: $(CH_3)_3C-$

-Bz : $-CH_2$-Phenyl

-Et : $-CH_2CH_3$

-Me : $-CH_3$

$-P_R$: $-CH_2CH_2COOR$

-Ph : -Phenyl

Arabic numerals refer to compounds for which an experimental procedure will be found in Chapter 5, and Roman numerals refer to other compounds or diagrams.

SUMMARY

This dissertation describes the design and synthesis of a number of porphyrin derivatives designed to model the active sites of the haemoproteins, particularly the natural oxygen-carrier, myoglobin. Previous models, which are reviewed in the text, have usually been derivatives of meso-substituted porphyrins. In contrast, the porphyrins used in this work were of the natural etio-substituted types, for these are potentially closer mimics of the enzymes' prosthetic groups.

Methods are given for the synthesis of porphyrins having two, three, or four propionate sidechains to carry the additional superstructure of the model systems. High yielding routes were developed using dipyrromethene precursors which are now available in large quantities. Several classical procedures in pyrrole chemistry have been improved. The carboxyl functions of the propionic acids have been used to attach a variety of "bridges" of atoms across the face of the macrocycle. The iron adducts of some of these bridged porphyrins have been characterised, and the reaction of the ferrous complexes with oxygen followed by visible spectroscopy.

Further developments are described utilising porphyrins having propionate substituents in which a differentiation has been achieved; this allows the synthesis of porphyrins having a bridge and also a sidechain containing an imidazole derivative, to function as a model for the proximal base in myoglobin. Finally, the preparation of a symmetrical doubly-bridged porphyrin is detailed: the iron complexes of these new ligand types were examined. The ferrous derivatives were treated with carbon monoxide and with oxygen to investigate their ability to model myoglobin as oxygen-carriers.

Extensive structural studies have been undertaken using proton n.m.r.. The use of deuteriopyridine as a solvent for porphyrin n.m.r. experiments has been examined. It is shown that this solvent has several advantages and that the resultant spectra are readily assigned. A study has been made of the

effect of the porphyrins' ring current on the chemical shifts of the protons attached to the bridges. In addition, lanthanide shift reagents and relaxation (T_1) measurements were used to probe the detailed structural features of the bridged porphyrins.

An attempt is made to unify the results of published model studies in the myoglobin field on the basis of the mechanism of oxidation of ferroporphyrins to their ferric derivatives. This seeks to rationalise the differences which exist between those models derived from porphyrins substituted at the meso positions and those which have no such substituent.

Many of the methods developed for the synthesis and structural analysis are of general applicability in porphyrin work aimed at understanding of the haem enzymes, and suggestions are made concerning future research possibilities.

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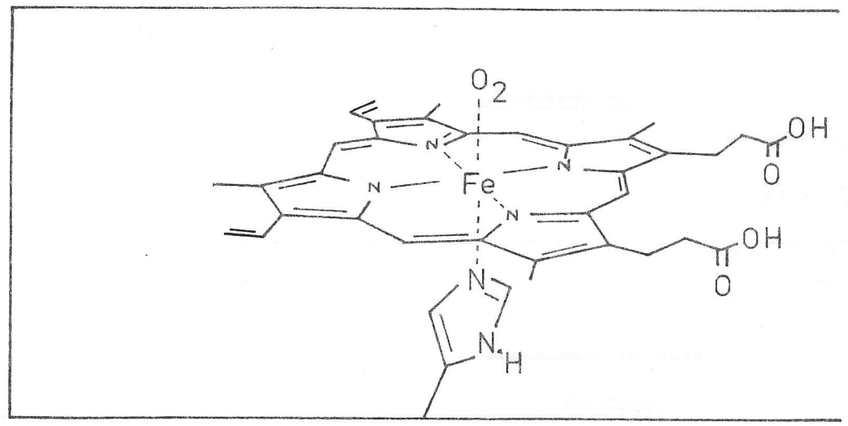


Figure 1 Myoglobin

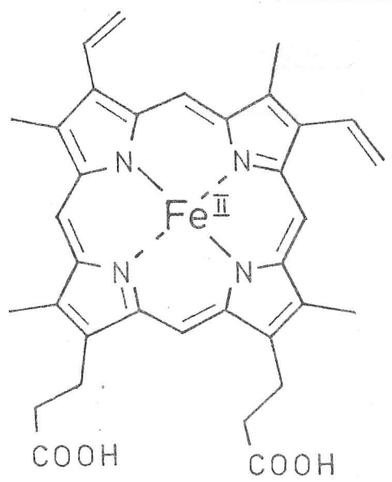


Figure 2 Ferroprotoporphyrin IX

CHAPTER ONE

a) Introduction

The interaction of molecular oxygen with biological systems is of fundamental importance in the maintenance of life. Nature has developed molecules such as haemoglobin, which combine reversibly with oxygen, to allow its transport through organisms, as well as a range of enzymes and cofactors to utilise it [1]. For example, cytochrome oxidase mediates the conversion of oxygen to water, and this reaction drives the respiratory chain. The monooxygenases and dioxygenases catalyse the introduction of oxygen into organic substrates, yielding derivatives having either one or two oxygen atoms incorporated per molecule of atmospheric oxygen consumed [2].

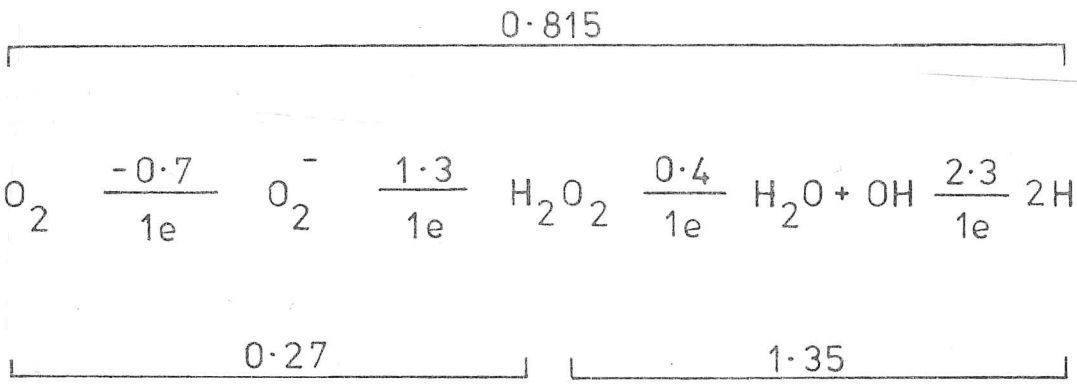
In each category of reaction, a central theme is the use of a haem prosthetic group having an iron atom contained in a planar four-coordinating ligand, a porphyrin; although iron is not the only metal involved in oxygen usage, nor haems the only catalytically active iron compounds. A principal aim of biochemistry is to understand how structure is related to function, and since the haemoproteins have such a diversity of function they have been the focus of much research.

The oxygen transport and storage proteins, haemoglobin (a tetramer) and myoglobin, have yielded most information so far, largely owing to the X-ray analyses of M. F. Perutz and J. C. Kendrew [3, 4, 5]. These showed that the active site consists of an iron (II) atom coordinated by protoporphyrin IX and a histidine residue of the globin, as shown schematically in Figure 1.

Other haemoproteins, for example catalase, peroxidase and the b class of cytochromes also have ferroprotoporphyrin IX (Figure 2) at their active sites; while some, like the a and c classes of cytochromes, contain a porphyrin of modified structure. The biosynthesis of these moieties has been thoroughly investigated [6] and many attempts made to understand the mechanisms, at the molecular level, of their actions in vivo [7]. These mechanistic studies

have proceeded in three related paths, each having a different emphasis.

Firstly, some workers consider the oxygen of chief importance and have examined its reactivity in terms of its redox chemistry and bonding [8]. Thus, a significant feature of the redox potentials for oxygen at pH 7 in water is, that while the overall four-electron process of conversion to water is thermodynamically favourable, the first one-electron step is energetically unfavourable [9]:



Redox Potentials in Volts.

A second approach has been to consider the properties of oxygen, as modified by coordination to a metal centre, and has led to studies of a huge range of metal-oxygen complexes [10, 11, 12, 13]. In a recent review [14], L. Vaska pointed out that "nearly all transition metals bind dioxygen" but that the mode of binding varies. In particular, there has been considerable speculation about the geometry of the metal-oxygen bond and, in the case of iron, whether the bonding is best expressed as Fe (II) - (O_2) or Fe (III) - (O_2^-) [15].

Thirdly, there have been experiments concentrating on the other ligands attached to the iron, the porphyrin and protein, which modify the redox properties and can alter the chemistry which might be expected in simple iron-oxygen adducts. Nature has chosen to vary the ligands, rather than the metal, in order to give a range of functions to the haemoproteins and it is of interest to ascertain to what extent changes remote from the iron can affect its reactivity [16, 17].

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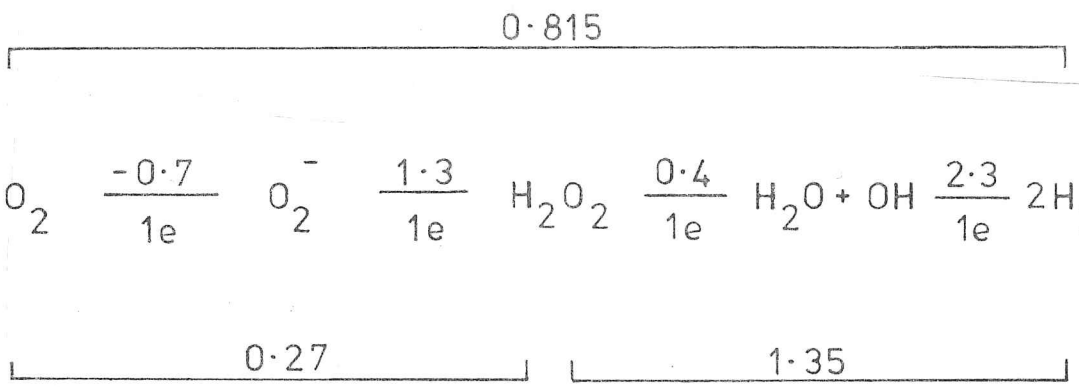

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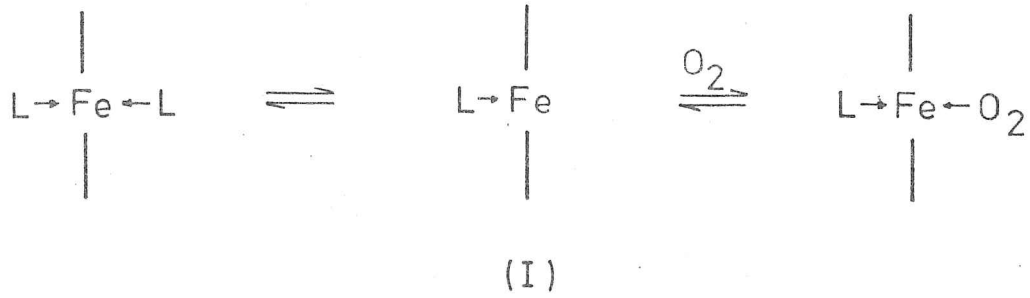
This latter bias is the one which will colour this thesis, and is the province of the organic chemist. The aim is to model the action of the haemoproteins by making simple iron porphyrin derivatives designed to duplicate their functions. If it is possible to mimic enzyme action to the extent, for example, of being able to carry out oxidation at specific unactivated carbon atoms or to use oxygen as part of an efficient fuel cell, then the rewards will be very great. The complex task of unravelling these natural systems has already attracted enormous research effort. Even with the restriction that our model systems should be iron porphyrins, the range is immense and we may feel, with R. J. P. Williams [18], that "the easy adjustment of geometric and electronic states of iron and porphyrin, of iron ligands and of near neighbours of the iron porphyrin makes for a problem in co-operative interactions which has far too many parameters for our understanding. It may be that our appreciation of enzyme action will fall short of full knowledge....."

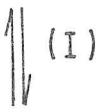
b) Model Studies

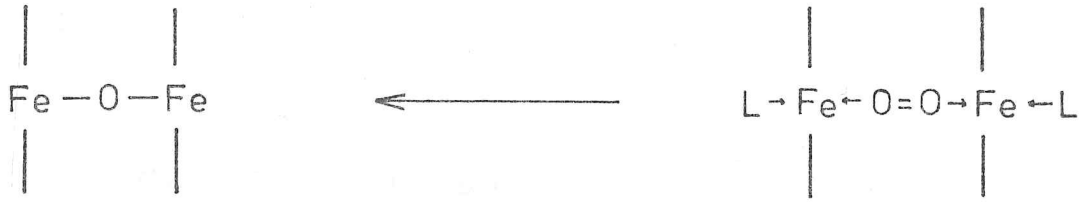
Early model studies in this field tended to confirm the pessimistic view, for it was soon discovered that even the process of reversible oxygen attachment to haemoglobin or myoglobin, in which no chemical change occurs, is remarkable; for ferroprotoporphyrin IX alone in solution is rapidly autoxidised to the ferric state on the addition of oxygen [9], although the rate can be lowered by addition of excess pyridine [19], a coordinating base. The product of this irreversible oxidation was shown to be a μ-oxo dimer [20] and the reaction can be understood in terms of the sequence of Figure 3 [21]. The reaction follows a complicated kinetic path, suggesting that multiple stepwise processes occur.



(I)

Figure 3





(II)

μ-oxo Dimer

L = a π-bonding ligand

Fe = a haem

Two molecules of the five-coordinated iron species (I) are implicated in one of the steps [22]. An important deduction was that if the dimerisation reaction could be slowed or prevented, the oxygen-binding would become reversible; and this led to later successful model studies [23]. The inability of a monomer to irreversibly oxidise may be considered a consequence of oxygen's poor one-electron oxidising power, in contrast to its two-electron reactivity. Iron chemistry is characterised by the instability of the (hypothetical) dioxygen-bridged dimer (II), unlike similar cobalt entities, which can be isolated and are not always subject to irreversible oxidation.

In retrospect, it is possible to understand the success of early model studies such as polymer-embedded haems [9] or in the solid state [24], in terms of their rigidly holding the porphyrins apart. The natural system, with its

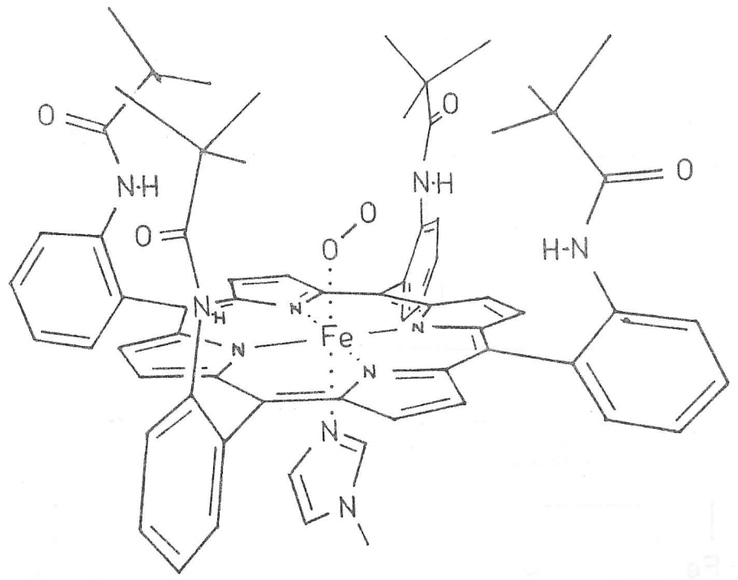


Figure 4 "Picket-Fence Porphyrin"

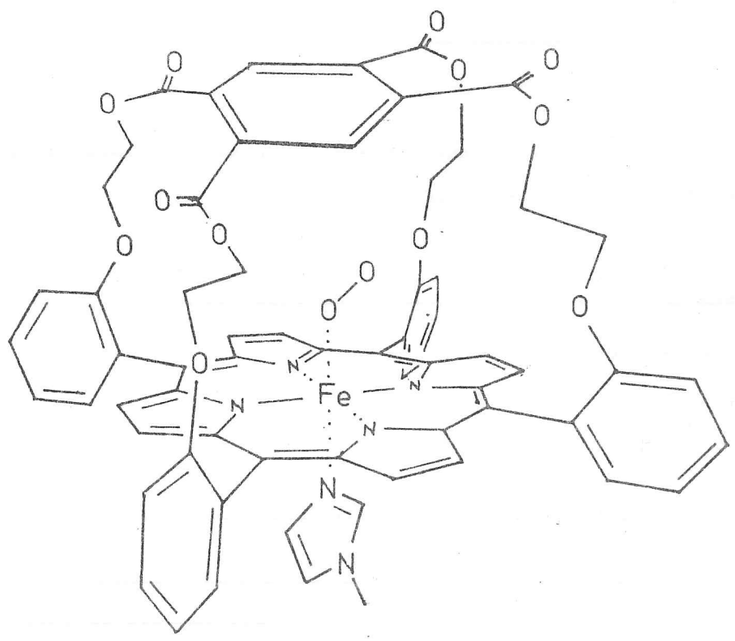


Figure 5 "Capped Porphyrin"

isolated haem in a protein cavity in the globin, also displays this feature.

There is only one example of a fully characterised model compound, that of J. P. Collman's group [21], whose "picket-fence" porphyrin oxygen complex has been examined by X-ray crystallography [25], as well as by analysis and spectroscopy. Their approach to the problem was to block one face of a porphyrin ligand by attaching to it bulky groups which would, however, leave sufficient space for oxygen to penetrate to the metal, as shown in Figure 4. A similar approach has been reported by J. E. Baldwin's group [26], who synthesised a porphyrin "capped" with a phenyl ring under which oxygen might shelter, as shown in Figure 5.

Each of these models, by preventing the unfavourable bimolecular reactions because of the steric bulk of the ligand, reversibly bind oxygen at 25°. Experiments have shown that the simple expedient of cooling a solution of a ferrous porphyrin will slow the autoxidation sufficiently to allow a study of the iron-oxygen adduct [27, 28, 29]; so only models which survive unoxidised at 25° can be said to have greater stability than ferroprotoporphyrin IX. It should be noted that even the natural system is not stable indefinitely, as oxidation to methaemoglobin types occurs. Nature employs a reductase system to restore the functional ferrous species [30].

A plethora of other model systems based on the idea of preventing dimerisation has now reached the literature, ranging from polymer supported haems [31, 32] to micelle isolated ones [33] and those in thin films [34]. T. G. Traylor's group have produced a series of "tailed" porphyrins [35] such as that shown in Figure 6. These models only bind oxygen at low temperatures, but have allowed experiments on the kinetics of the oxygenation process [36, 37], without the necessity for the presence of an excess of an axial base like imidazole in solution. These, and similar studies [38, 39, 40, 41], lead to a further understanding of the influence of the proximal histidine in haemoglobin, and open the way to the attachment of a range of ligands needed to mimic the cytochromes.

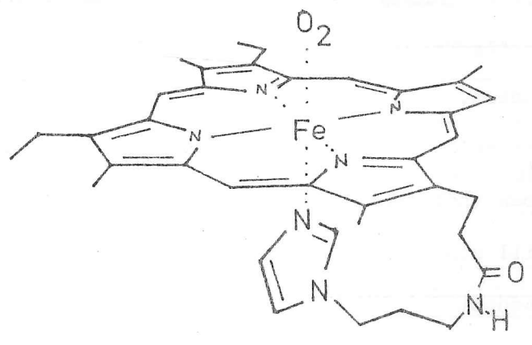


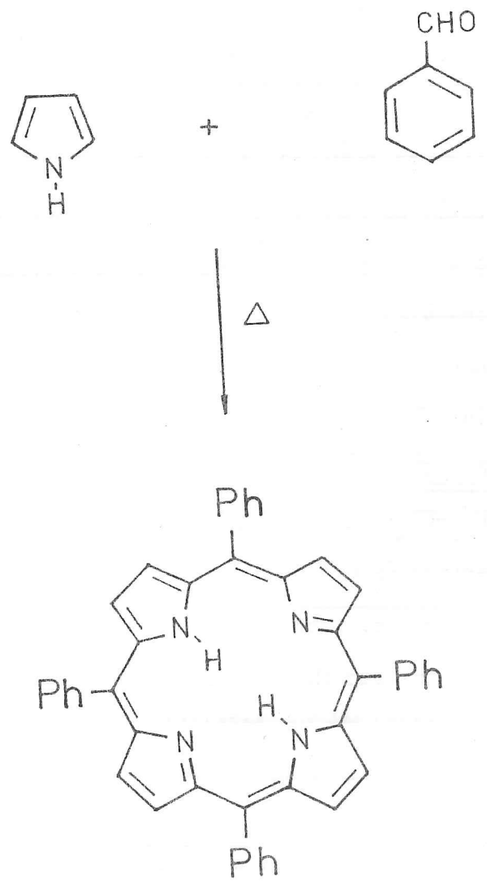
Figure 6 "Tailed Porphyrin"

T. G. Traylor *et al.* also reported a more ambitious model which combined features of the capped porphyrin with those of the tailed series [42]. However, the synthesis was very long, and did not allow them to pursue the oxygen-binding properties of these molecules.

At the outset of the experimental work described in later chapters, it was possible to list some objectives for a second generation of myoglobin models, under three general headings.

First, it was considered desirable to base future syntheses on porphyrins resembling haem as closely as possible. That is, in particular, the meso-substituted porphyrins of the picket-fence and allied types should be replaced by those having alkyl substituents at the β-positions of the parent pyrroles and unsubstituted connecting methene bridges (the meso positions). The motivation was threefold: to interfere as little as possible with haem's electronic and substituent structure, to reduce the molecular weight of the molecules required (the meso positions conventionally carry phenyl groups of no particular utility) and to increase the solubility of the intermediates and products. The meso positions are the sites of the maximum electron density in both the HOMO and the LUMO of the conjugated ring and hence the preferred sites of both electrophilic and nucleophilic attack [43]. In addition, many metalloporphyrins are more reactive than their metal-free counterparts and it is likely that Nature employs the meso positions in electron transfer to the haem, in an outer-sphere process [44, 45].

Secondly, any new route to candidate active-site models should be flexible and high-yielding to allow fine-tuning at late stages in the synthesis until the desired properties are achieved. Earlier syntheses of models have tended to accept low-yielding routes in return for experimental convenience, and have also sacrificed flexibility in order to achieve a particular target quickly.



(III)

meso-Tetraphenylporphyrin.

Figure 7

Thirdly, it would be attractive to build up a repertoire of functionalised porphyrins which might be of use later as starting points for the synthesis of mimics of molecules as diverse in function as the P_{450} cytochromes, chlorophyll or vitamin B_{12}. There is a large body of pyrrole chemistry to be exploited in such a project.

Clearly, any new myoglobin model will no longer be required to prove the principle of oxygen-binding, which has been amply demonstrated, but should lend itself to spectral comparisons with the natural systems. This will be particularly important if future studies (for example of oxygenases) are to include oxidised or reduced porphyrin rings (that is, radical cations or anions) as intermediates, since in these the electronic structure will be expected to differ in meso-substituted or non-substituted types [46].

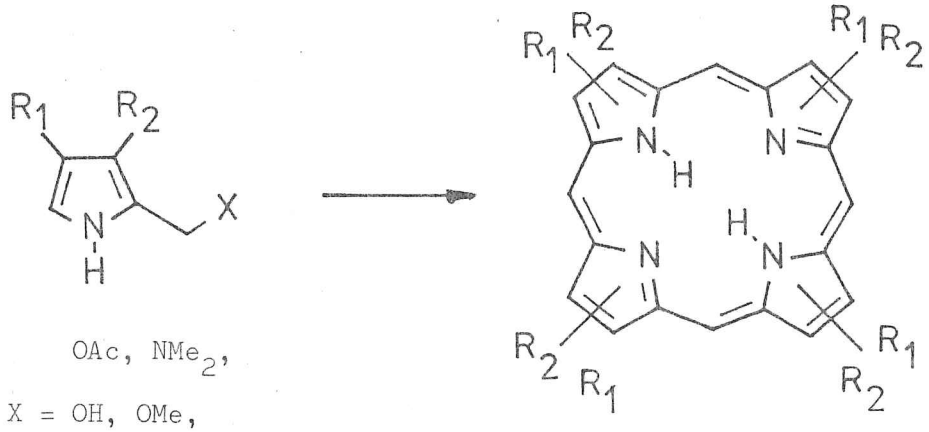
A further consideration is that the visible spectra of metalloporphyrins change according to substituent type, and although a good theoretical model of these effects is available [47], the comparison with haemoproteins will be easier in etioporphyrin models.

c) Porphyrin Synthesis

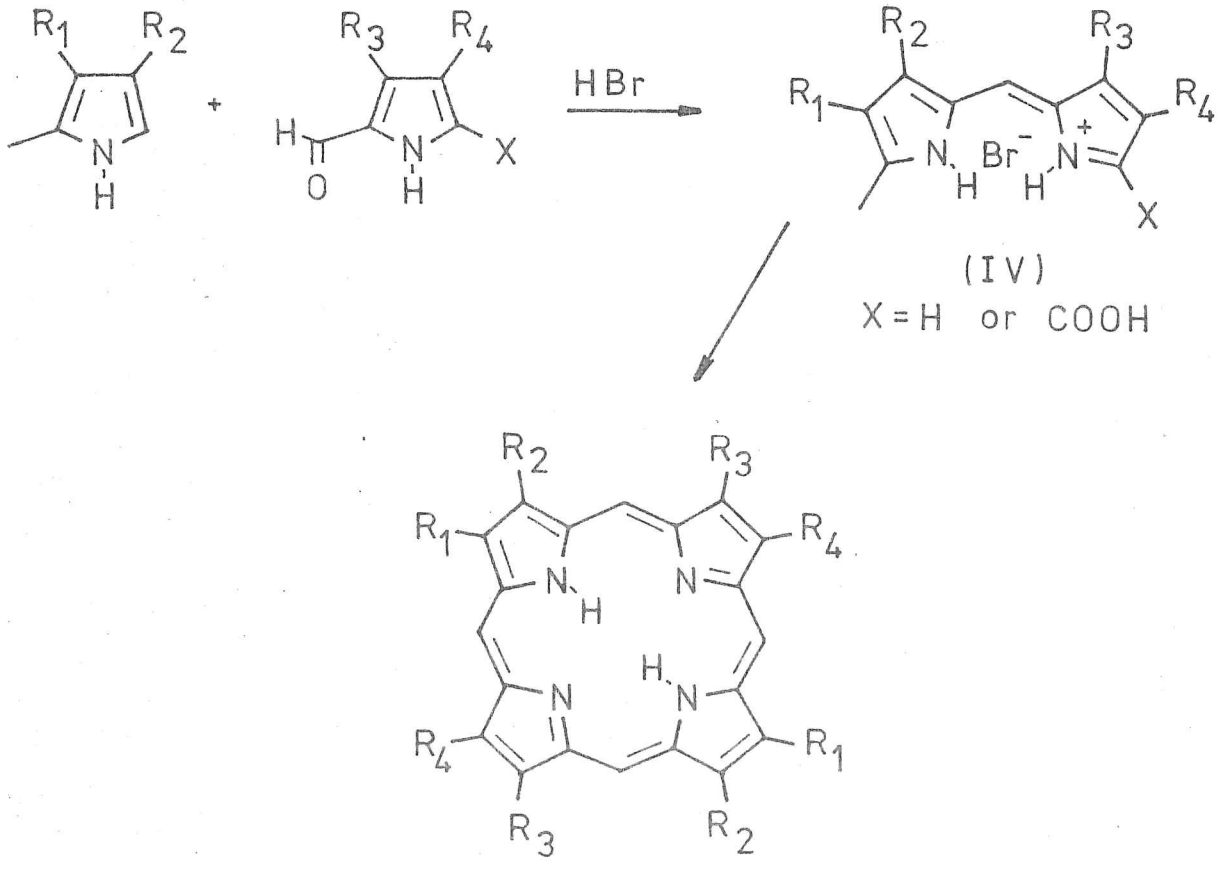
There are several recent reviews of porphyrin synthesis [48, 49, 50, 51], work building on the mammoth efforts of the Fischer school [52]. In general, procedures have been developed to exploit the symmetry features that the target molecule possesses. For example, meso-substituted porphyrins with four-fold symmetry are particularly easy to prepare in one step from an aldehyde and pyrrole, as in a recipe [53] for the parent tetraphenyl porphyrin (III), which is shown in Figure 7.

This simplicity has been responsible for the extensive experimentation with these types. Self-condensations of mono-pyrroles were the basis of many early syntheses, although mixtures result unless $R_1 = R_2$ in the

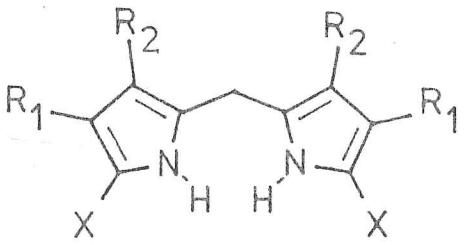
reaction scheme:



A more useful approach is the synthesis that now bears Fischer's name, which is best suited to porphyrins having a centre of symmetry and has as key intermediate a dipyrromethene like (IV):

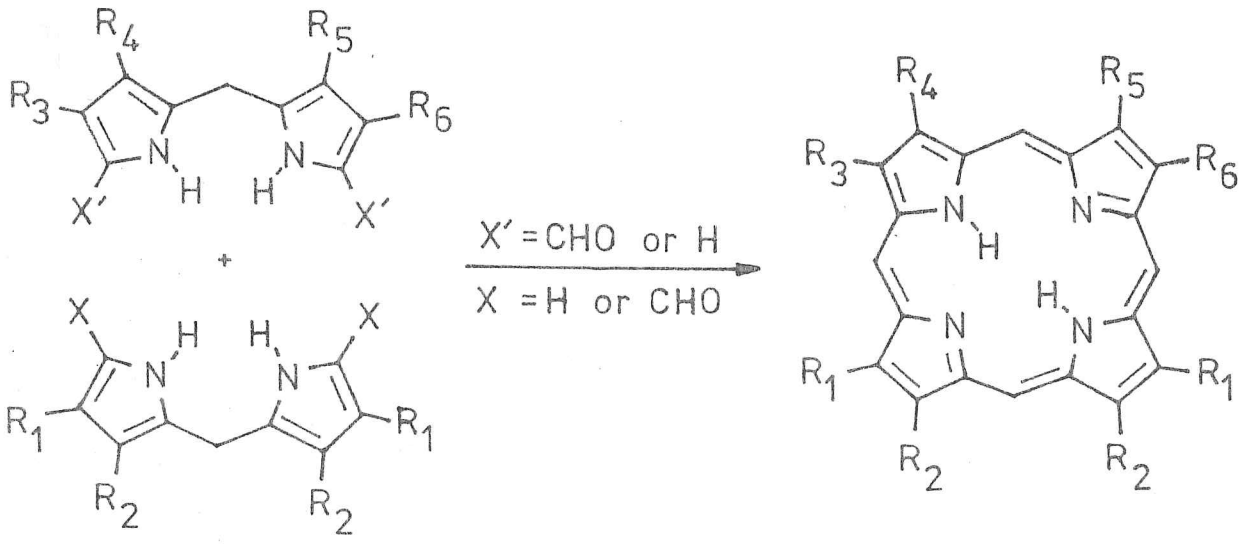


Natural porphyrins usually have one dipyrrolic unit with a mirror plane of symmetry, and for their synthesis a significant development was the "MacDonald" route [54], which is based on a dipyrromethane of structure:



X = H
or X = CHO

When this is condensed with a complementary functionalised dipyrromethane, which can carry four different β-substituents without giving a mixture of products, a porphyrin having the important protoporphyrin IX substituent pattern can result:



Finally, for unsymmetrical molecules, either of the above general approaches can be modified successfully, and both have been important in recent biochemical studies [6]. Other syntheses using, for example, dipyrryl ketones, have also found some applications.

With such an armoury of methods, it seemed likely that whatever porphyrin were to be chosen for elaboration in the present work, a suitable synthesis could be designed.

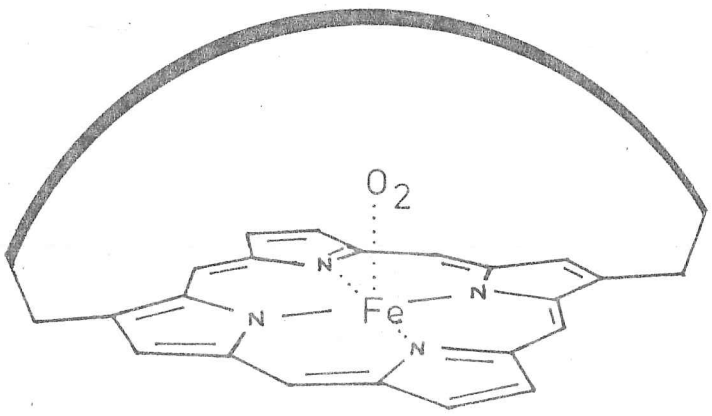
CHAPTER TWO

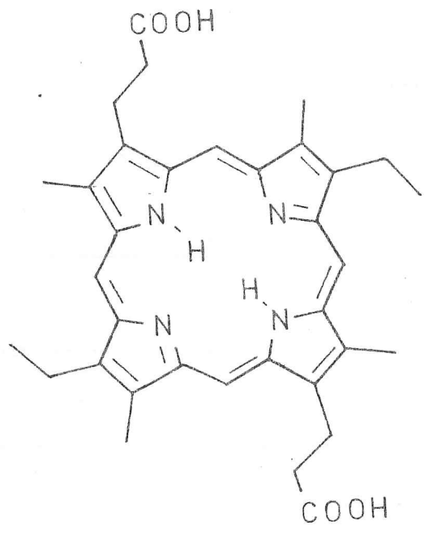
a) Choice of a target porphyrin

The objectives of the project were fourfold:

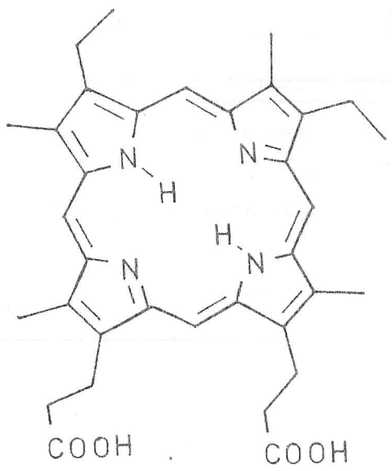
1) To discover the minimum requirements for reversible room-temperature oxygen-carrying.
2) To design a flexible and high-yielding route to candidate molecules.
3) To interfere as little as possible with Haem's electronic structure.
4) To allow developments to molecules which might mimic the cytochromes or chlorophyll.

With these aims in mind, it was possible to plan the first group of new myoglobin models. It was decided to block one face of the porphyrin, while allowing free access by oxygen to the iron atom at the centre of the macrocycle. This might be achieved by constructing a "bridge" across the face. Clearly such a bridge requires at least two points of attachment to the porphyrin and these should avoid the meso positions to maintain the etio substitution pattern. The bridge should be symmetrically disposed to ensure that the central part of the cavity so formed, above the metal, would be blocked to the bimolecular reactions which are evidently so unfavourable. Such a target was also likely to be the easiest to synthesise. The bridge had to be large enough not to hinder the approach and coordination of the oxygen to the metal, which defined the general features of the molecule:





Mesoporphyrin II



Mesoporphyrin IX

Figure 8

At the outset, when there was no precedent for such a bridged system constructed by attachment to a pre-formed porphyrin, it appeared likely that a number of bridging procedures would have to be attempted. Therefore, propionate sidechains were chosen to provide a versatile anchorage point for further elaboration. The propionate group is present in protoporphyrin IX, and thus many standard pyrrole syntheses have been developed including it[49,52].

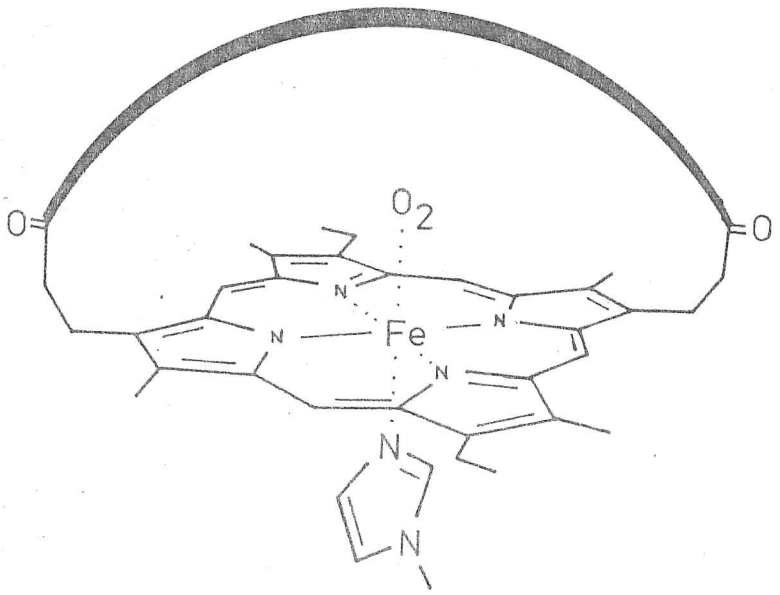
Further substituents around the porphyrin periphery were then a matter of choice. Ideally, it might be attractive to use some free position to attach the extra base which acts as sixth ligand for the metal: alternatively, these positions could be filled with carboxylic acid residues in the hope of promoting water-solubility. In either case, the experimental procedures would be more complex than needed solely to fulfil the bridge-building rôle; so, it was initially decided to restrict substituents to methyl and ethyl groups. Mesoporphyrin II [52] (Figure 8) was chosen as a suitable goal for a large-scale synthesis as it met the requirements of having two suitably disposed propionate chains, and had several other attractive features.

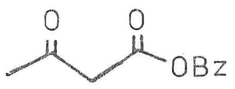
Firstly, its centre of symmetry would allow a classic synthesis of the Fischer type: indeed, H. Fischer himself synthesised it in his investigations into the structure of haem [52] and modern versions of his method were expected to afford large quantities with relative ease, a consideration of prime importance when the porphyrin would become merely the starting-material for further elaboration.

Secondly, it is related to mesoporphyrin IX (Figure 8), which is itself known to be a molecule whose iron adduct will function as a prosthetic group in myoglobin [55], despite the replacement of the vinyl groups in the haem by ethyl groups. Vinyl groups are difficult to synthesise on porphyrins, owing to their acid instability, and they make the macrocycle more susceptible to photo-induced oxidation; so they are to be avoided in a model system, particularly when it is known that their contribution to the functioning of the enzyme is small.

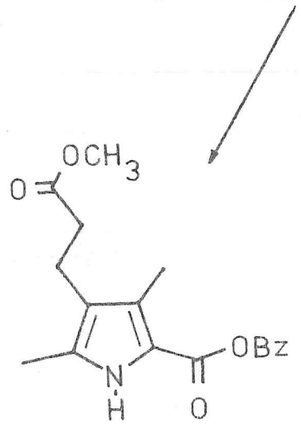
Finally, the ethyl groups were expected to be of value when studying the proton n.m.r. of the compounds, for the triplet of the terminal methyl would occur [56] at 1.9 δ, an uncrowded chemical shift region, and hence providing a standard for integration and identification. The alternative choice of a bis-methyl or bis-ethyl pyrrole for the unfunctionalised positions would, in contrast, be expected to give a more complicated spectrum of overlapping signals.

In most studies of oxygen-binding, an imidazole base has been employed as the axial ligand [21, 26], and N-methyl imidazole is the customary choice, since it has similar properties to histidine as a coordinating base, without the acidic NH proton which in model studies complicates the acid-base behaviour [29], and which in Nature is hydrogen-bonded away from the haem. The plan in the current work was also to employ N-methyl imidazole, in the hope that it would, on steric grounds, preferentially coordinate away from the bridged side of the porphyrin, and would block the otherwise open face. This is the behaviour that was observed with the picket-fence model, and gives a refined view of the target:

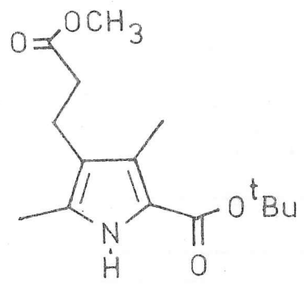




(1)

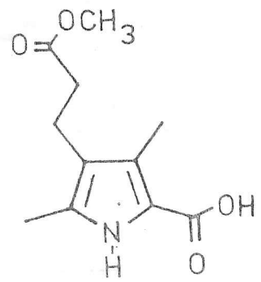


(2a)



(2b)

H_2



(3)

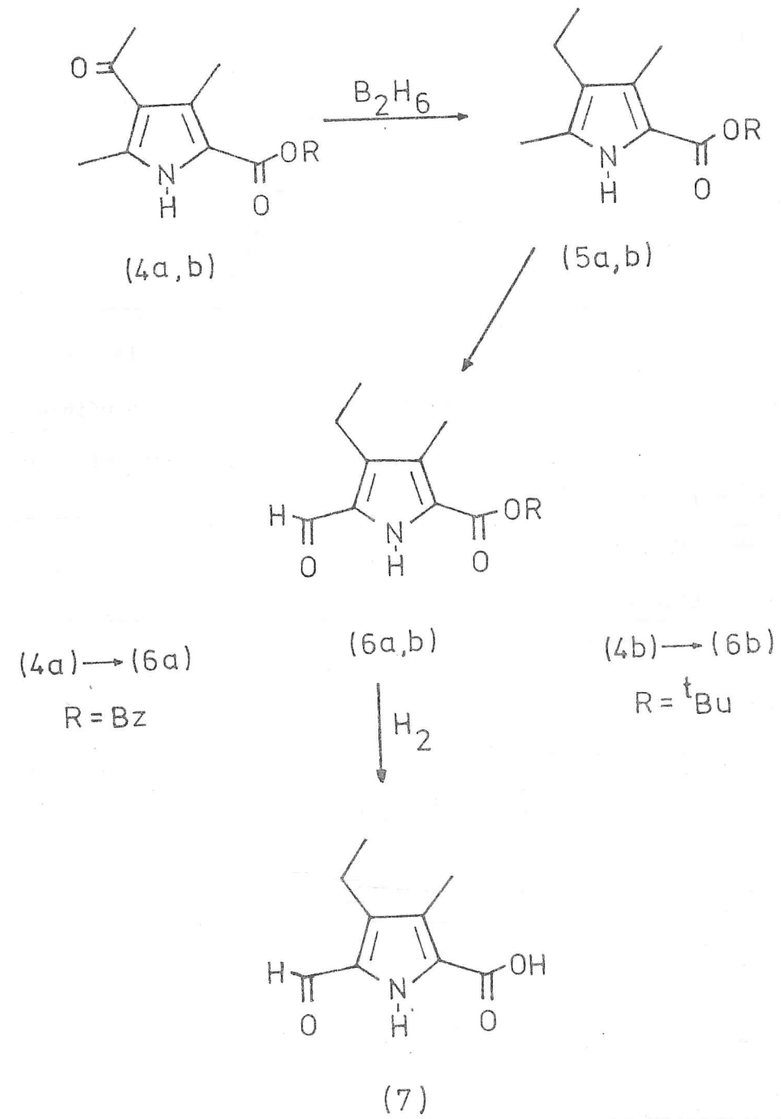
Scheme 1

The synthetic task could now be split into two parts. One was the construction of the basic porphyrin, and the other the attachment to it of whatever was found to be necessary for the closure of a bridge of the desired size. This large-ring synthesis could be attempted in a variety of ways. For example, acetylenic coupling [57], the Dieckmann [58] and acyloin [59] reactions, and the synthesis of lactams and lactones [60] have been successful in other cases.

b) The synthesis of mesoporphyrin II

Although mesoporphyrin II is a known compound [52], no high-yielding route to it had been published, since modern interest was confined to a study of the spectra [56, 61] of this non-natural isomer. It appeared likely that about 10 g of the material would be required for bridge-building studies; in fact, over 30 g have been prepared in the course of this work. This is considerably larger than the scale of porphyrin synthesis for biological or labelling studies and an efficient route was desirable. Several approaches were therefore examined.

The synthesis followed the dipyrromethene "Fischer" plan, condensing a pyrrole-α-aldehyde with an α-free pyrrole, followed by dimerisation in hot formic acid, with bromine as oxidant [62] (see Chapter 1 Section (c)). In this case, the α-free pyrrole precursor (3) was readily available (Scheme 1), by a modified Knorr reaction with benzyl acetoacetate (1) [63], followed by removal of the benzyl group of the resultant pyrrole (2a) by hydrogenation. Under the acidic conditions of the subsequent dipyrromethene formation, the pyrrole was expected to decarboxylate, avoiding an isolation of the unstable α-free pyrrole and improving the yield. This concept may be extended by using the tert-butyl ester as protecting group during the Knorr reaction, producing (2b). This loses iso-butylene and carbon dioxide in strong acid, and is therefore also equivalent to an α-free pyrrole.



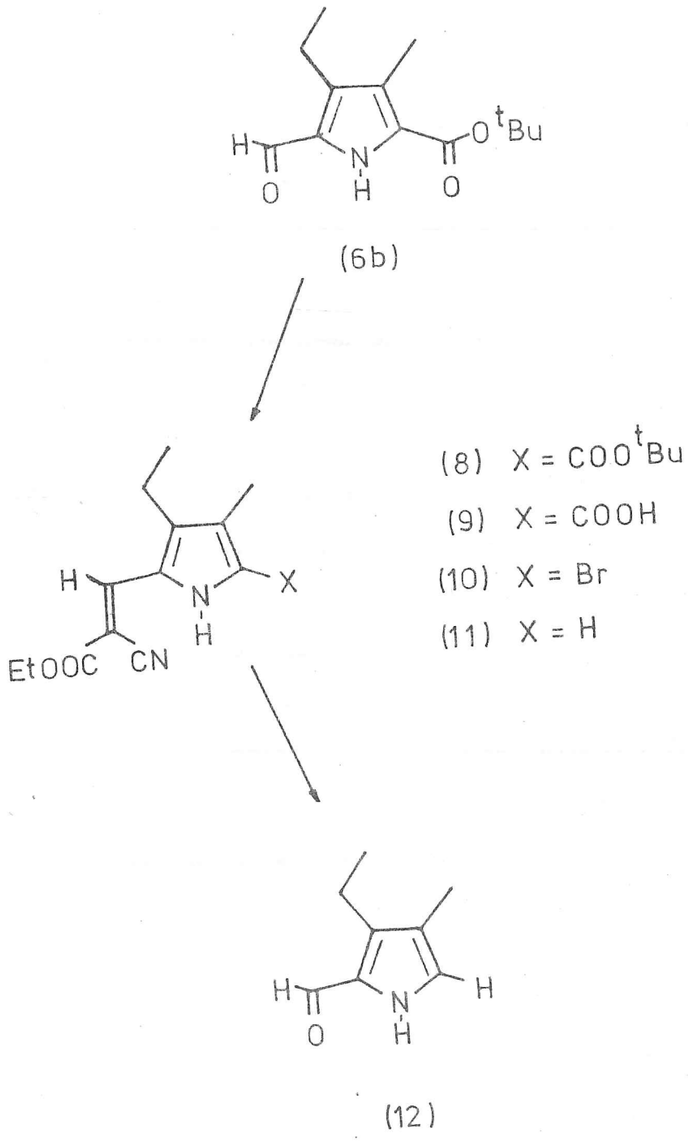
Scheme 2

Both these approaches were tried, and either gives good yields of dipyrromethene. The chosen plan of including the propionate group with the α-free pyrrole made it unnecessary to have it present in an oxidation to a pyrrole-α-aldehyde; a reaction in which it was expected to be more sensitive than the alternative, outlined below, wherein only alkyl groups are subjected to the oxidant. The yield from (1) to (3) was 40%, the loss of material occuring at the Knorr reaction, which seems to admit no improvement.

The overall yield of mesoporphyrin II possible is critically dependant on the synthesis of the pyrrole-α-aldehyde used, and for that reason, several routes were investigated. The choices available include those shown in Scheme 2, which gives aldehydic esters (6a) and (6b).

In either case, the initial Knorr products (4a) or (4b) were reduced to the corresponding pyrroles (5) by the very useful reaction developed by H. W. Whitlock and R. Hanauer, using diborane generated in-situ in tetrahydrofuran [64]. There was then a choice of oxidation step to the aldehydes. The use of lead tetraacetate in acetic acid is mild and efficient and was obligatory for (5b), because the alternative (using sulphuryl chloride as oxidant) which is applicable to the acid-stable benzyl ester (5a), would cleave the tert-butyl group. This latter method is more attractive on cost grounds, but was anticipated to give lower yields using the published experimental conditions [65]. In fact, by modifying the method, using dichloromethane rather than ether as solvent and working up in aqueous tetrahydrofuran, the yield was found to be comparable to that of the lead tetraacetate reaction, at 90%.

At least three alternative aldehydes might have served for the dipyrromethene formation. One was (6b), where, as was the case with (2b), the tert-butyl group would disappear as iso-butylene during the reaction. This simplest alternative was not found to be as useful as hoped, however, since the aldehyde was only crystallised with difficulty and could not be isolated

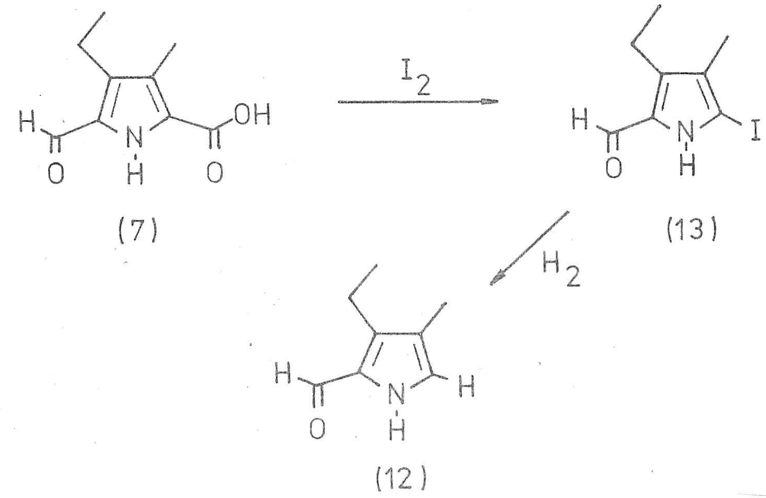


Scheme 3

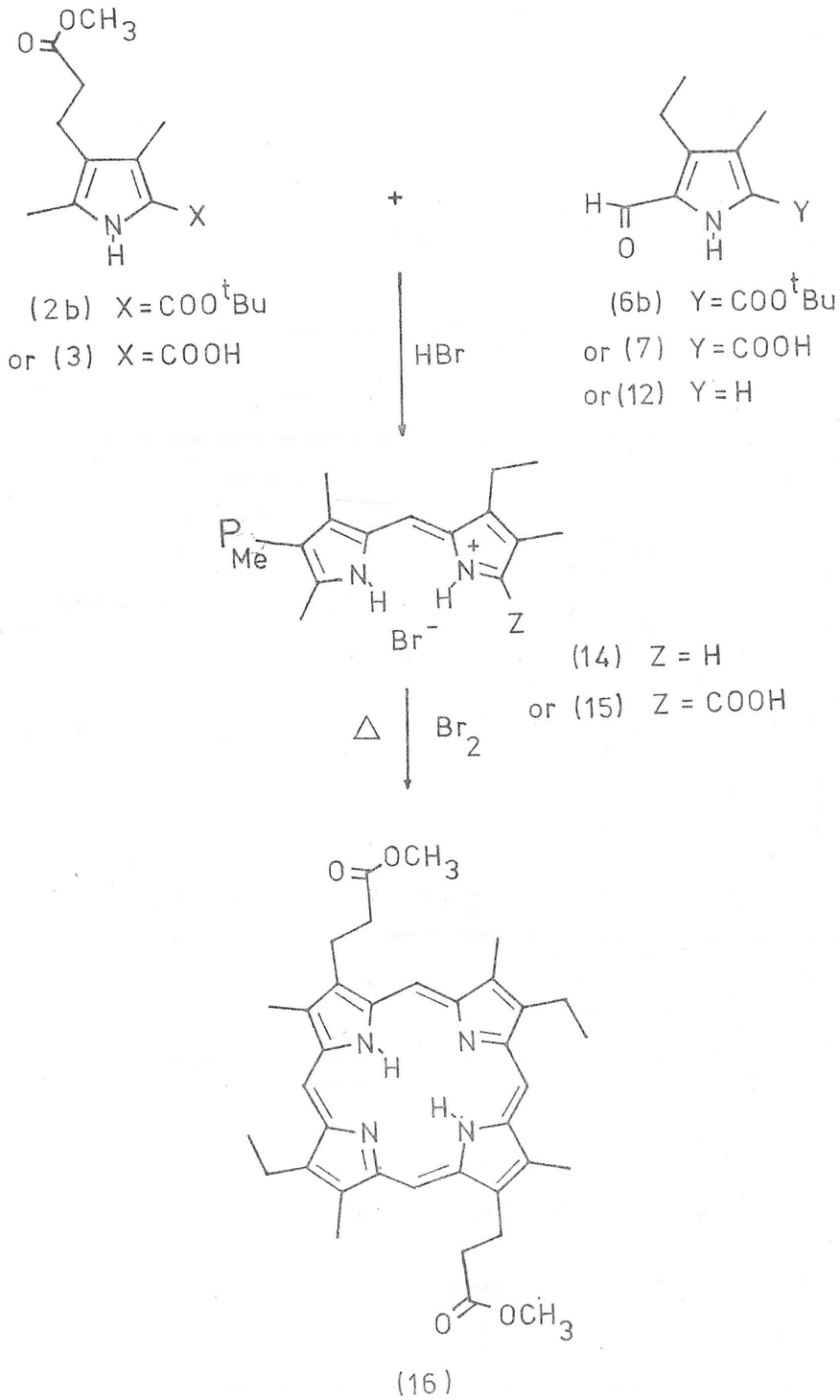
in good yield from the oxidation step unless a substantial proportion was scavenged as the Knoevenagel adduct [66] (8). This, in turn, could be converted as shown in Scheme 3 to the aldehyde (12).

These high-yielding reactions involved treating the masked aldehyde with trifluoroacetic acid to remove the tert-butyl group, giving (9), which was treated with bromine to afford (10). After hydrogenolysis to (11), the aldehyde could be deprotected in base to produce the desired (12). The use of ethyl cyanoacetate to provide the Knoevenagel adduct allowed this last step to proceed under milder hydrolysis conditions than would have been applicable with a dicyanovinyl protecting group [52, 66]. The replacement of the commonly used malononitrile reagent with ethyl cyanoacetate was suggested by J. B. Paine III, following his work with methyl cyanoacetate as a protecting group for pyrrole aldehydes [67].

An alternative source of the α-free aldehyde (12) involved conversion of (7) to (13) by iodination, followed by hydrogenolysis:



Although this was an attractive proposition, and crude yields of (13) were high when a two-phase iodination in chloroform and aqueous sodium

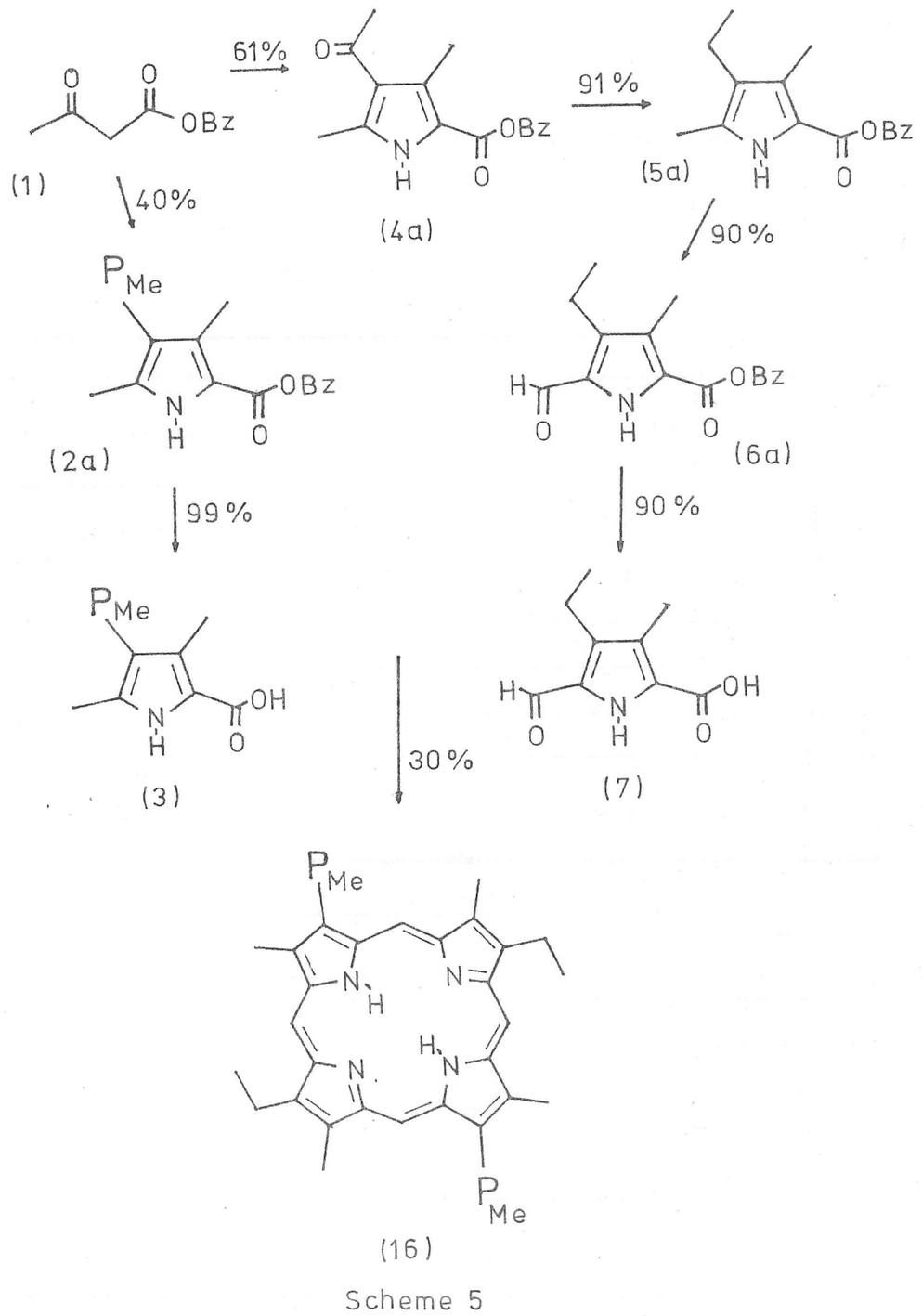


Scheme 4

tri-iodide was carried out, it was found that the hydrogenolysis step was not reliable. Even when Adam's catalyst was used, it only proceeded reproducibly on crystalline iodo-aldehyde, and crystallisation was, unfortunately, wasteful of material. The conversion of (7) to (12) could only be accomplished in about 20% yield on all but the smallest scales.

Both (6b) and (12) are useful dipyrromethene precursors, and (7), the product of hydrogenolysis of (6a) (Scheme 3) was also considered. Each aldehyde was taken through a porphyrin synthesis, as shown in Scheme 4. Reaction with the previously prepared (2b) or (3) and hydrobromic acid gave the alternative dipyrromethenes (14) and (15), both nicely crystalline and isolated in about 80% yield. It was anticipated from the literature on dipyrromethenes [49] that they might be difficult to purify because of their reportedly poor chromatographic behaviour. It was found, however, that thin-layer chromatography on silica gives good separation of these and related compounds provided that the solvent system (chloroform / methanol is suitable) contains about 5% of acetic acid to keep them in their protonated form. Column chromatography was possible, too, but in general proved unnecessary. Indeed, the whole synthesis up to the porphyrin has been carried out without resort to chromatography.

Cyclisation of either (14) or (15) to the porphyrin was accomplished by heating them at reflux in formic acid containing two molar equivalents of bromine, and was most successful when, in addition, the solvent contained about 10% of acetic anhydride to remove the last traces of moisture. Bromination proceeds whether the dipyrromethene is α-unsubstituted or carries a carboxylic acid group in that position (the intermediate brominated compounds have been isolated), and hence both (14) and (15) give the porphyrin (16), the yield falling from about 38% with the former to about 27% with the latter. This simplified procedure is a modification of H. Fischer's original method which was pioneered by K. M. Smith [62]. There is no need to isolate brominated intermediates and the overall yield is better than in the earlier



Scheme 5

work.

In assessing the best sequence for large scales, the yields over all steps, the cost and the time taken had to be weighed. In particular, the preparation of the aldehyde (12) in good yield on large scales (50 g) was difficult, in contrast to the excellent results possible with small quantities. S.G. Hartley observed [68] that hydrogenolysis of (6a) to (7) was more consistently successful on 0.2 M scales when the aldehyde, which was usually contaminated with small amounts of sulphurous impurities from the sulphuryl chloride oxidation, was filtered through a bed of Raney nickel in dry tetrahydrofuran prior to being stirred with hydrogen and palladium. Early attempts to scale up that step had been thwarted when it was found that large (and expensive) quantities of catalyst were required to overcome the poisoning due to the sulphur. After the nickel treatment, however, 1 g of 10% palladised charcoal was sufficient to convert over 50 g of aldehyde in a reasonable time.

Another significant finding was that there was no need to isolate dipyrromethenes in the conversion of the two pyrrolic components to porphyrin. The procedure could be carried out in formic acid in an overall yield of 30%. In summary, the best choice is given in Scheme 5, where typical yields for the scaled-up reactions are given. The pyrrole reactions have been applied to between 0.2 and 1 molar batches and the final step was conveniently used to produce 5 g quantities of porphyrin.

The overall yield to mesoporphyrin II, isolated as its dimethyl ester, was 29% from (2a) and 23% from (4a), both available in multi-molar amounts from the Knorr reactions.

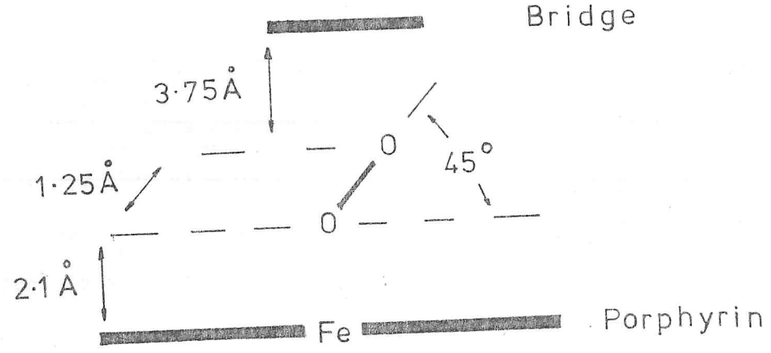
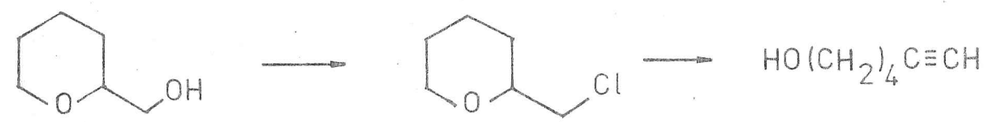


Figure 9

c) Bridging reactions

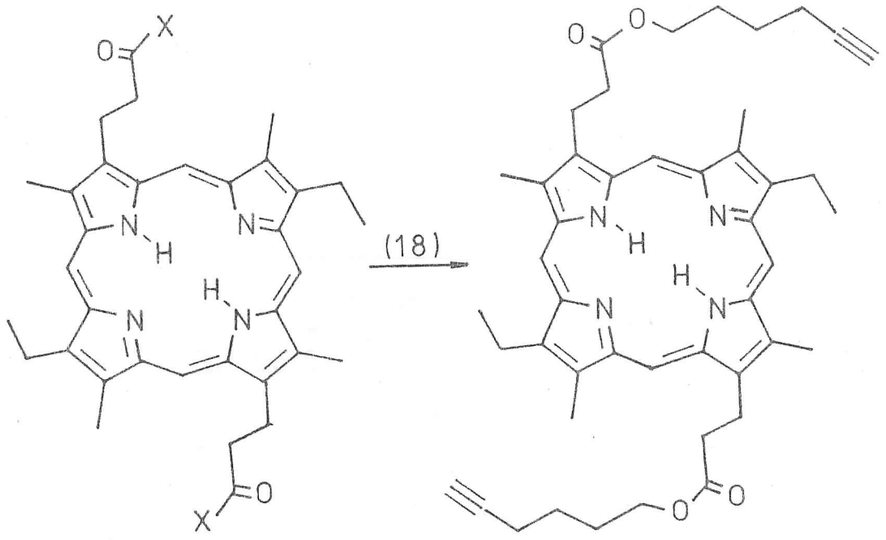
The syntheses attempted for the construction of the protective bridge were largely dictated by the desire to mimic as closely as possible the size of the cavity in myoglobin. From the known X-ray structure of picket-fence porphyrin [25] and the distances in myoglobin from the coordinated oxygen to its nearest neighbours [69], it was possible to lay down general size criteria for the bridge, assuming that oxygen-binding would be similar to that found before. The distances that were thought to be critical are shown in Figure 9. Inspection of a series of proposed structures by construction of their respective C. P. K. space-filling models [70] allowed a judgement to be made of the likely space available and hence of the correct bridge size. The conclusion reached was that fourteen atoms should connect the two carbonyl groups of mesoporphyrin II.

The first method tried for the building of the bridge was the Eglinton modification of the Glaser reaction, the coupling of terminal acetylenes [71]. This was chosen because of its wide application in the closure of rings of all sizes, both strained and unstrained, and because coupling is possible under mild conditions. The acetylenic alcohol (18) required to provide the correct bridge size could be attached to the porphyrin via its acid chloride, and was readily made [72] from the commercially-available tetrahydropyran-2-methanol:



(17) (18)

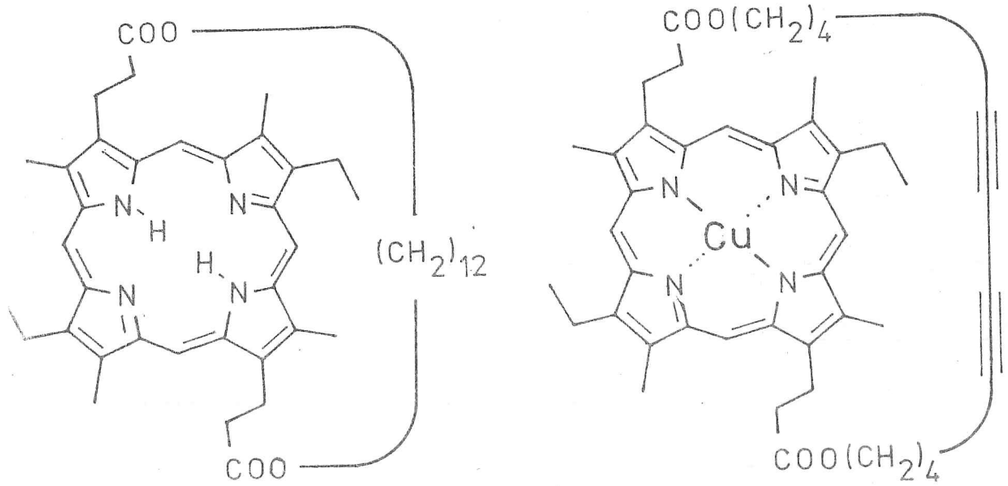
Conversion to the chloride (17) with thionyl chloride in pyridine, followed by elimination using sodamide in liquid ammonia, gave the alcohol (18) in 56%



(16) X = OMe
(19) X = OH
(20) X = Cl

(21)

Scheme 6



(23)

(22)

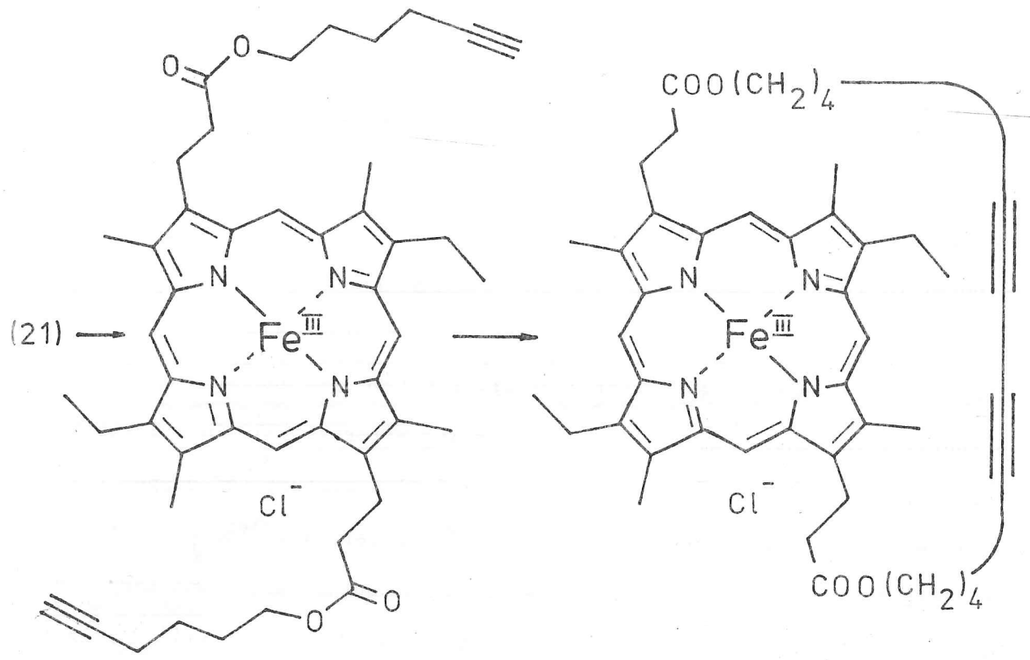
yield.

The reactions of Scheme 6 were then carried out. The porphyrin free-acid (19) was prepared by hydrolysis of its ester (16) in 6N hydrochloric acid, and this transformed with thionyl chloride to its bis acid chloride (20), which was not isolated, but immediately treated with the acetylenic alcohol to give the diester (21) in 85% overall yield.

The high-dilution conditions for cyclisation to a monomer product were easy to achieve with the porphyrin ester, for this was assisted by its low solubility in ether. By placing the starting material (21) in a Soxhlet thimble, it could be introduced into the reaction mixture (of copper (II) acetate in pyridine / ether) at a rate of 0.1 g per day. The product was obtained in 60% yield after chromatography. Structure proof was hampered by the presence of the copper, which had metallated the porphyrin during the reaction. The metal's spin broadened all the signals in the n.m.r. and precluded the use of that technique. However, analysis, infra-red, and visible spectra were all consistent with the proposed structure, and high-resolution mass spectrometry confirmed its composition.

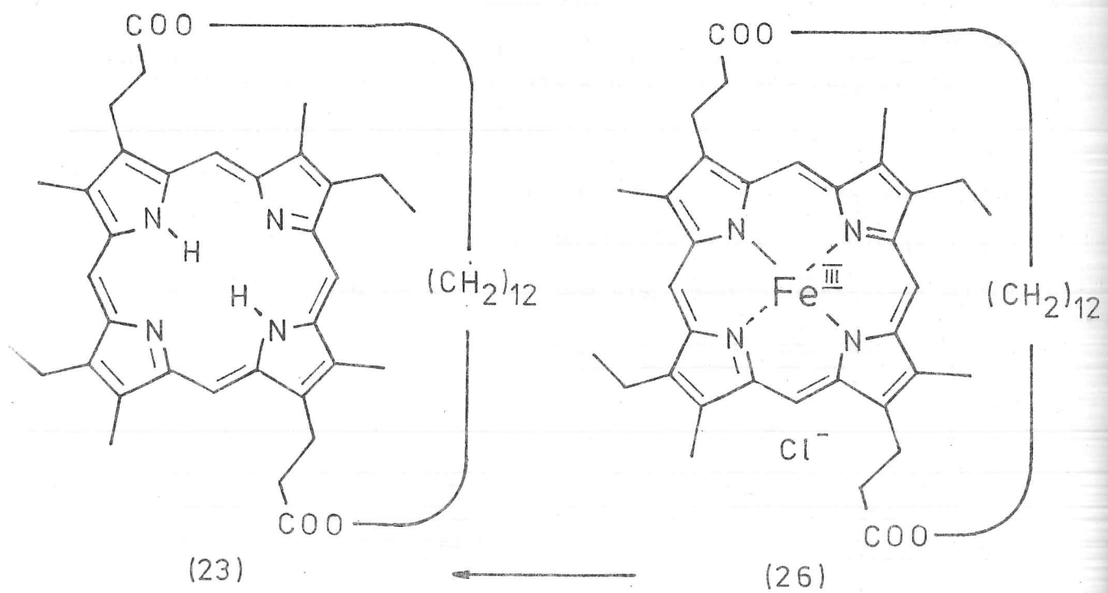
Further evidence of the success of the reaction came when the material was catalytically reduced to saturate the bridge, and then demetallated by repeated treatment with trifluoroacetic acid [73], which generated the diprotonated porphyrin, followed by neutralisation in dichloromethane and water. The metal-free porphyrin (23), after further purification, gave an n.m.r. spectrum in which, significantly, signals for the protons of the bridge appeared at higher field than in (21); the shift being due to the effect of the ring current in the macrocycle. The proton n.m.r.'s of the bridged porphyrins in general are of interest, and they are dealt with in Chapter 4.

Since the demetallation only gave a 40% yield, a more strategic approach to the desired iron-containing model (25) was used, that of Scheme 7.



(24) (25)

Scheme 7



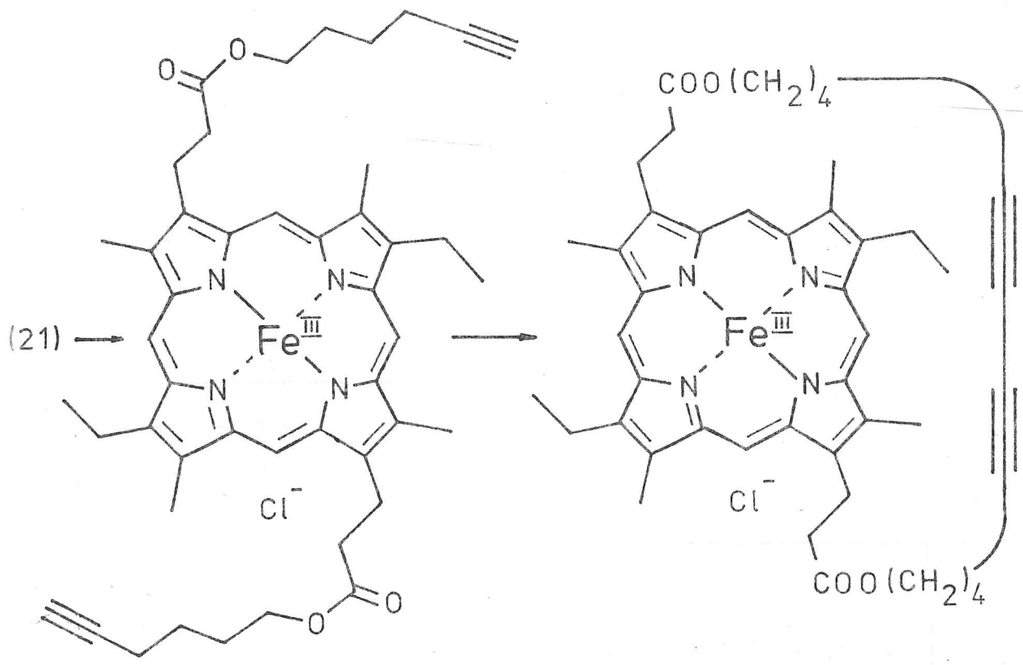
(23) (26)

Introduction of iron into the acetylenic diester (21) gave (24), and it was expected that the copper acetate reagent in the acetylenic coupling would not displace iron (III) from the porphyrin, since ferric chelates are stable to transmetallation [74]. This was borne out when (24) was cyclised and (25) obtained in 42% yield from (21).

Again, full structural proof was difficult, for the ferric system also has residual spin which broadens n.m.r. signals. However, after a similar sequence to that with the copper analogue, namely, catalytic reduction to (26) (itself of interest as a potential oxygen-carrier) followed by a standard demetallation [75], the compound could be shown to be correctly cyclised. In particular, the metal-free material (23) was identical to that obtained previously.

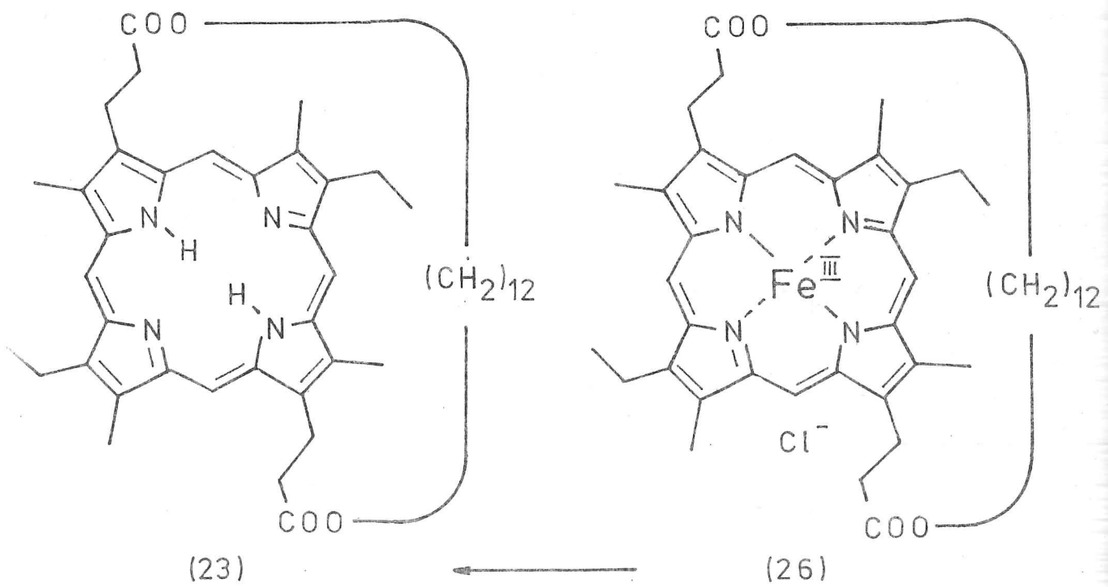
A second bridge-forming sequence was then explored. This had the double objective of providing some functionality (other than triple bonds) at the centre of the bridge, for possible future studies of appended ligands, and of giving an amide-linked bridge which would have greater stability (especially to acids) than the lactones of the previous target. The bridge-closure was carried out using the Dieckmann ester condensation [58], and the whole sequence used is shown in Scheme 8.

N-methyl caprolactam (27) was prepared from caprolactam in a slight modification of the literature procedure. It was reported by R. E. Benson and T. L. Cairns [76] that either O-methyl or N-methyl caprolactam can be made by treating caprolactam with dimethyl sulphate and, that while use of one equivalent gives the former, an excess will provide the latter. However, in the present work, it proved to be very difficult to complete the isomerisation of O-methyl (the kinetic product) to N-methyl unless the unwanted isomer was first isolated by vacuum distillation and then subjected to thermal isomerisation at its boiling point, in the presence of a small quantity of dimethyl sulphate.



(24) (25)

Scheme 7



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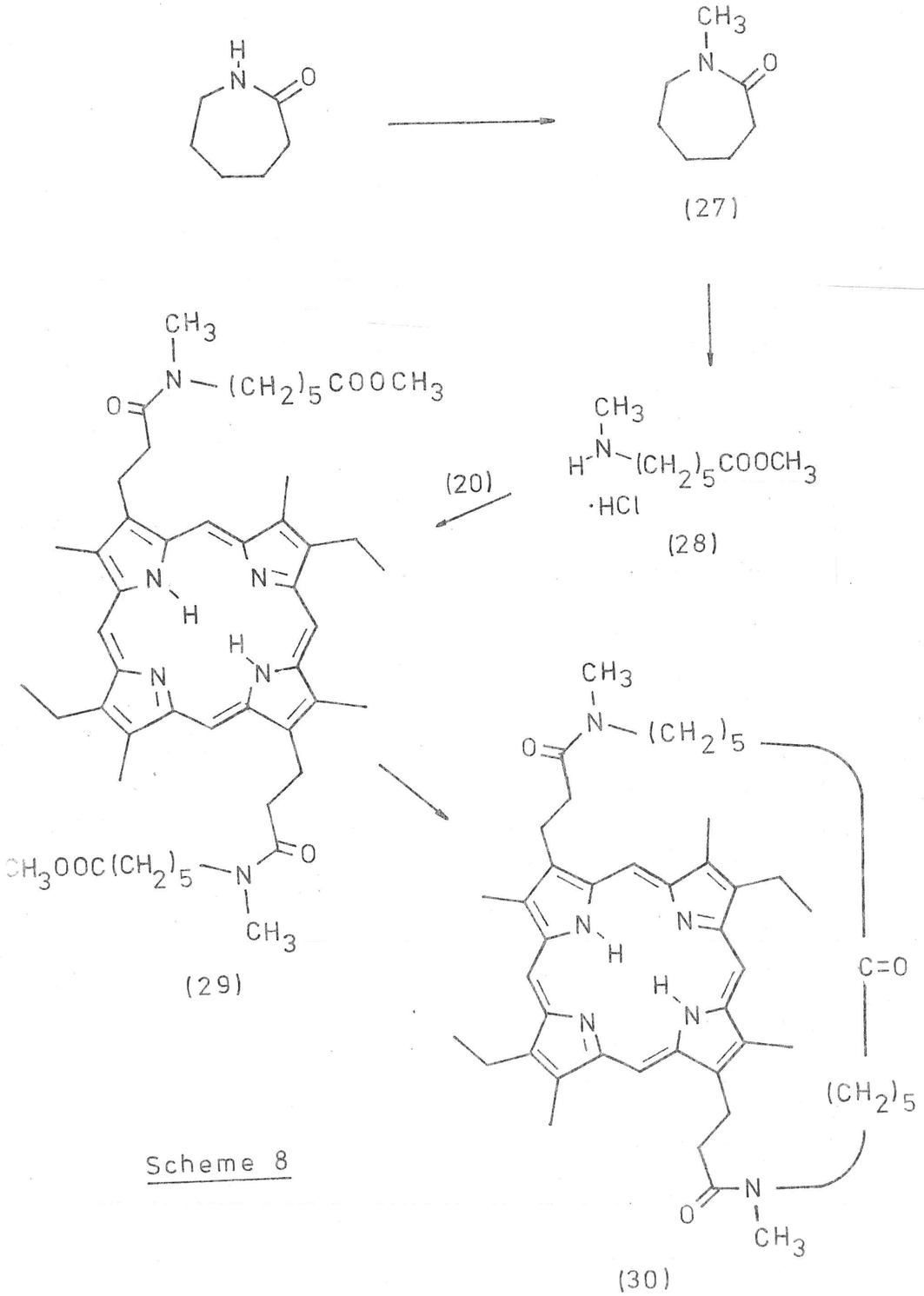
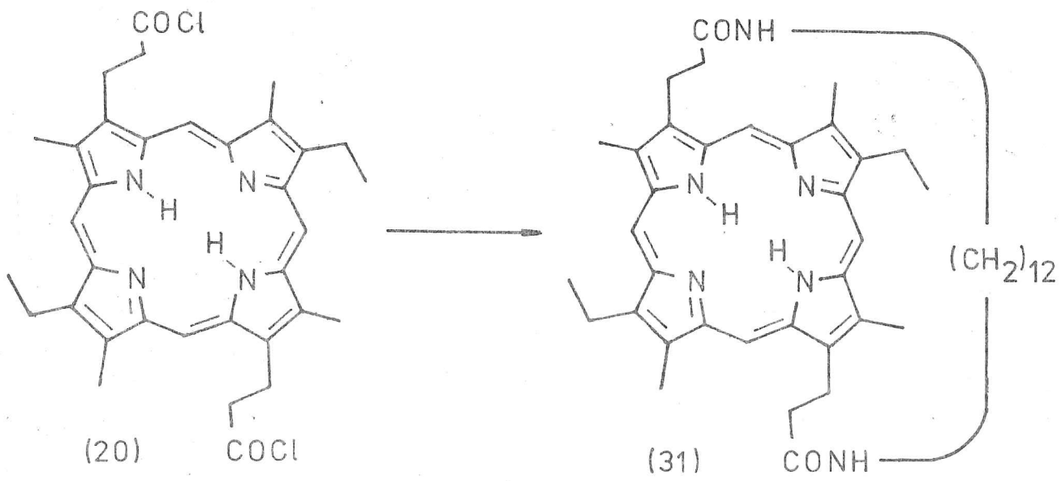


Scheme 8

Hydrolysis and esterification of (27) then gave the required amine ester (28) in moderate yield. Isolation of this product was difficult because of its ready reversion to N-methyl caprolactam, but could be accomplished by crystallising it from strong acid as its hydrochloride salt. Condensation with the porphyrin bis acid chloride (20) gave the bis amide (29), which was cyclised in the standard high-dilution Dieckmann manner [58], using potassium tert-butoxide in toluene. This proceeded in 28% yield to the keto bridged porphyrin (30). The intermediate having a methoxycarbonyl substituent α to the ketone was not isolated, and presumably suffered hydrolysis and decarboxylation during the work-up, which included chromatography on alumina, a reported method for effecting such a transformation [77].

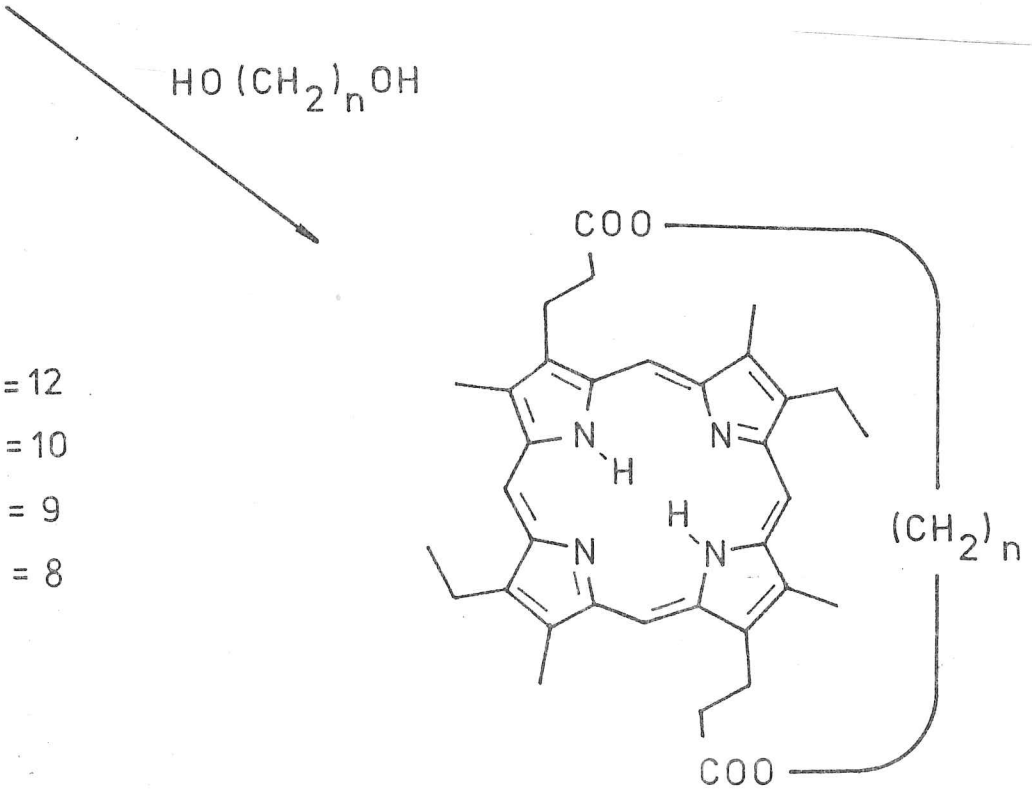
After achieving success in the above ring-closure reactions, it was felt that an even simpler approach to bridged porphyrins merited attention. It appeared likely that merely to mix the porphyrin bis acid chloride (20) with a diamine, might, at high dilution, give acceptable yields without undue polymer formation; a method similar to that used in other large-ring syntheses [78].

After some experimentation, it was found that in dry dichloromethane and with added pyridine, the reaction was quite straightforward:



When the total volume of solvent used was about one ml per mg of porphyrin, simple slow mixing of the diamine solution and the porphyrin solution gave yields of around 30% of the adduct (31), which was readily separated from polymeric byproducts by chromatography. In addition, when the diamine was replaced by a diol, the yields were better, reaching 50%, and a series of bridged species were accessible:

(20)



(23) n = 12
(32) n = 10
(33) n = 9
(34) n = 8

The increase in yield may be attributed to the lower basicity of the alcohols, which, unlike the amines, would not be expected to protonate in the acid produced during the reaction. It was not possible to add a stronger non-nucleophilic base to the amine reaction to circumvent this protonation problem, because when that was attempted decomposition of the acid chloride was observed, possibly via ketene intermediates.

When dodecane-1,12-diol was used, the product was the same bridged compound (23) which had been obtained from the demetallation of (22) and of (26). Lower homologues of this diol afforded (32), (33), and (34) which were of interest in connection with the influence of the bridge size on its distance from the porphyrin, and the resultant chemical shifts of its protons. The closer to the macrocycle the centre of the span is constrained to be, the further upfield the resonances in the n.m.r. appear (see Chapter 4).

A similar, independently conceived, approach to bridged porphyrins has recently been reported by H. Ogoshi and his co-workers [79, 80], who used a mixed anhydride to activate a porphyrin diacid to attack by diamine. These methods have already been exploited [81] for the construction of more exotic systems since the first publication of our results [82].

With this knowledge of bridge-forming reactions, it was then possible to predict that any desired system could be available, provided that the rings so formed were not strained and that the method chosen was compatible with other functionalities in the molecule. Therefore, a priority was to test the oxygen-carrying properties of the model compounds (25) and (26), and to determine what further modification might be needed.

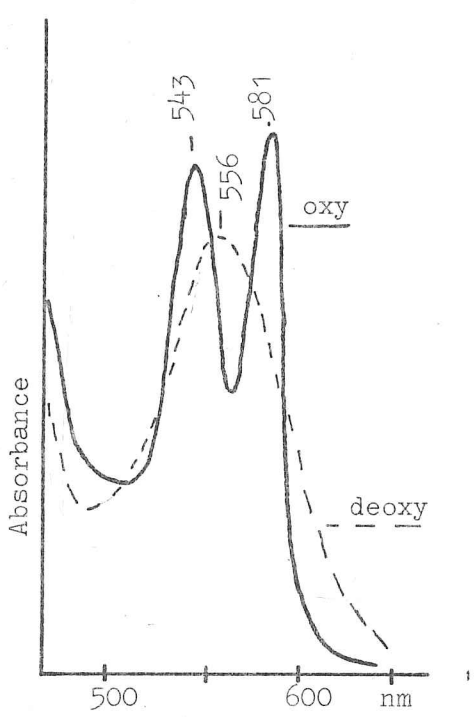
d) The reaction of singly-bridged models with oxygen

The reactions of metalloporphyrins may conveniently be followed by visible spectroscopy, as a large range of metals in a variety of oxidation states give characteristic, well-documented, spectra [74, 75]. Care must be taken, however, to ensure that spectra are recorded under comparable conditions of temperature, solvent, concentration, etc., if reliable interpretation of the often complex pattern of absorbance is to be made.

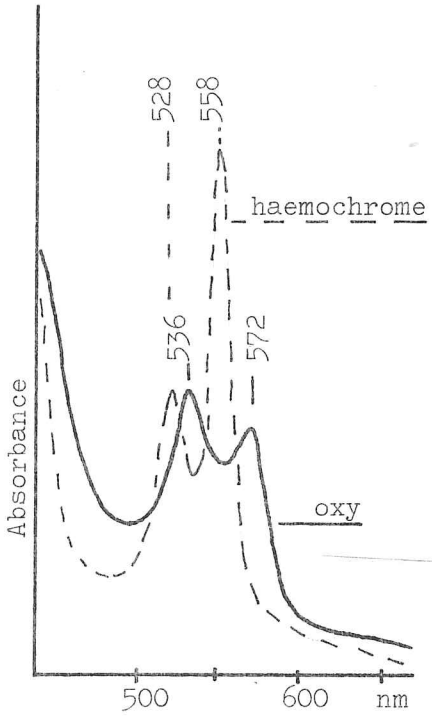
For the current discussion, the relevant spectra are those of ferric and ferrous porphyrins when other ligands such as N-methyl imidazole, oxygen,

and carbon monoxide are present. All these species have been extensively studied, and the results reviewed [74, 83, 84].

As a starting point, the spectra of myoglobin and ferroprotoporphyrin IX dimethyl ester may be compared [84, 85]:



(a) Sperm Whale Myoglobin
pH 7, 20° (Ref. 84)



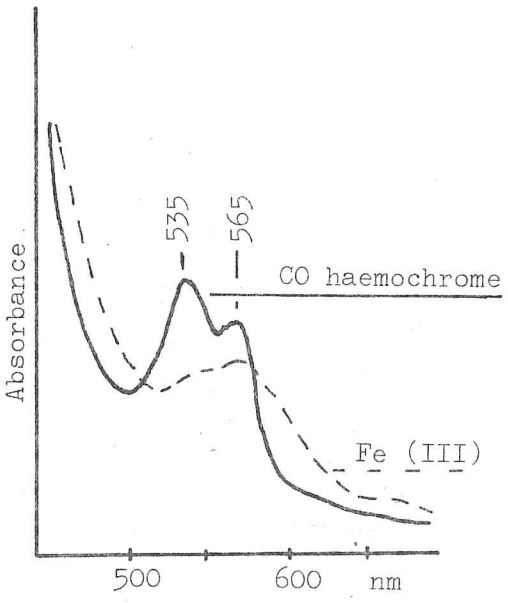
(b) bis-(1-butylimidazole) haem ester, oxygenated at -45° (Ref. 85)

Figure 10

The six-coordinate low-spin "haemochrome" spectrum (Figure 10 (b)), with two sharp peaks at 528 and 558 nm, is representative of Fe (II) porphyrins in which two π-donor ligands (such as pyridine or imidazole) are coordinated in the axial positions. The single peak shown by deoxymyoglobin (Figure 10 (a)) corresponds to a five-coordinated high-spin system with only one axial π-donor ligand.

On oxygenation, both the native myoglobin and the isolated haem become six-coordinate and low-spin, with the absorption maxima shifting to about 540 and 575 nm, and the relative intensities of the bands changing. It should be noted that the isolated haem is now only stable at low temperature (ca. -45°), so the precise absorption maxima, which show minor temperature variations, cannot be directly compared.

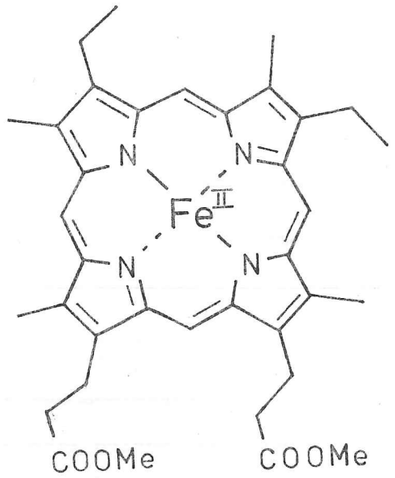
Two other species are relevant to a study of the reactivity of haems with ligands. One is the carbon-monoxy haemochrome produced when carbon monoxide is allowed to react with a haemochrome (replacing one of the axial ligands), or to displace oxygen from an oxygenated species like oxymyoglobin. The other is the Fe (III) haem, produced when a ferric compound becomes six-coordinated and low-spin. Examples of these types are well known, and their spectra are represented by those of Figure 11:



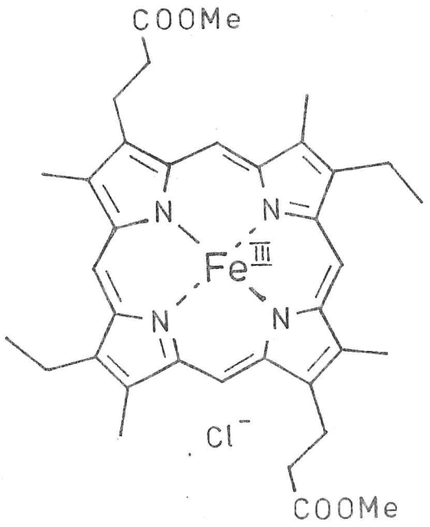
bis-(1-butylimidazole) haem ester as starting haemochrome (Ref. 85)

Figure 11

The precise absorption maxima again vary with the haem used — and even the shape of the curves may change when meso tetra-aryl porphyrins are studied. However, the literature provides examples of most of the types which will be met in the current work. Thus, J. E. Baldwin et al. report some relevant spectra for mesoporphyrin IX, which closely resemble those anticipated for

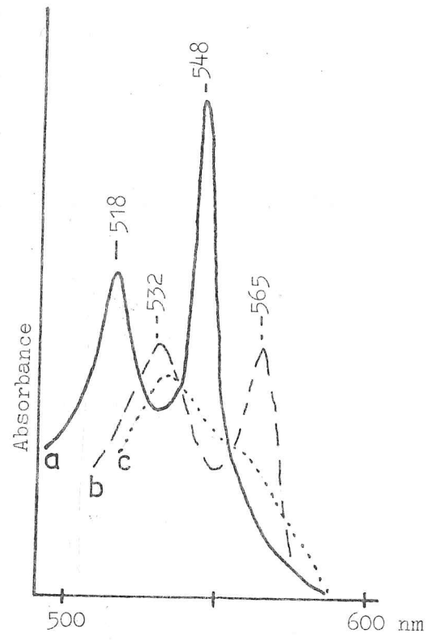


(V)



(16a)

a successful etio-porphyrin model [29]:

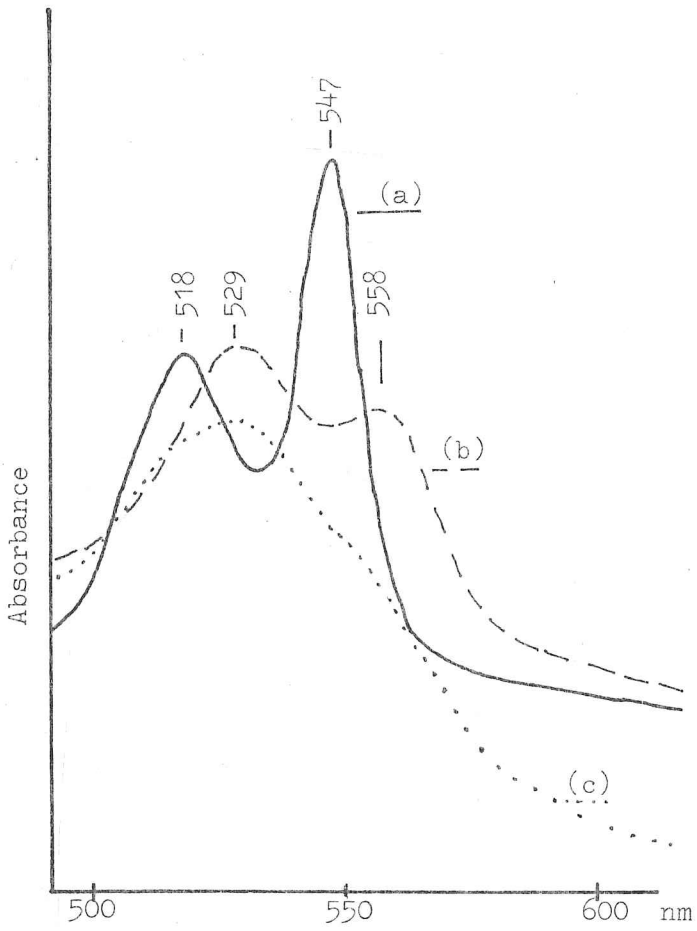


Compound (V) in CH_2Cl_2 at -50°
(a) haemochrome with N-methyl imidazole
(b) oxygenated
(c) oxidised (ferric)
(Ref. 29)

Figure 12

The haemochrome and oxygenated compounds differ only in detail from those of ferroprotoporphyrin IX — the replacement of the vinyl groups by ethyl groups causes a shift to lower wavelength which is readily understood from the theoretical model of M. Gouterman [47] — and the shapes of the maxima are similar. Again, it should be noted that the oxygenated species is stable at -50°, and warming produces the ferric form.

In order to have a representative set of spectra, the compound (16a) was prepared, the iron adduct of mesoporphyrin II. This would provide a basis for comparison with the bridged model compounds (25) and (26). In addition, the experimental methodology could be developed with this readily-available material rather than the more precious model compounds. Typical spectra are shown in Figure 13.



Compound (16a) in acetone at 20° (after reduction)

(a) haemochrome with N-methyl imidazole

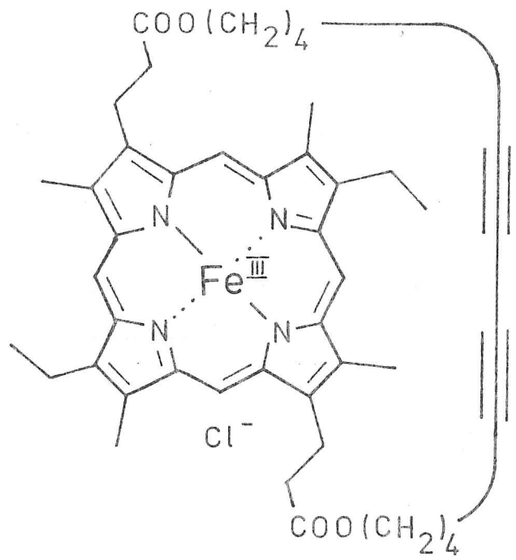
(b) carbon-monoxy haemochrome

(c) oxidised (ferric)

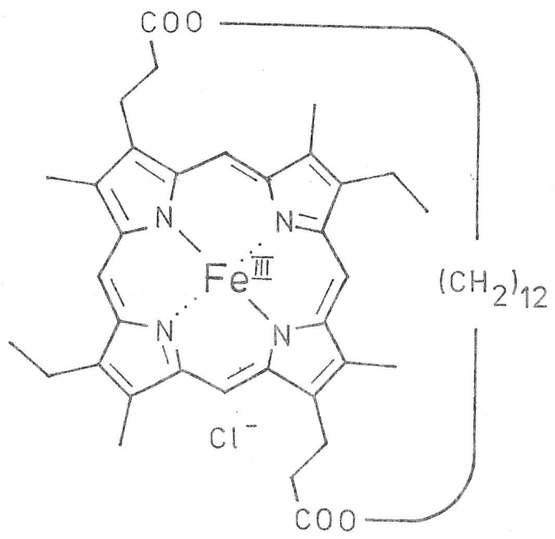
Figure 13

This example depicts the results in acetone when reduction was accomplished with aqueous sodium dithionite at 20° (see experimental section). The haemochrome was obtained when N-methyl imidazole was injected into an oxygen-free sample of the Fe (II) porphyrin. Subsequent addition of carbon monoxide gave the characteristic change as the carbon-monoxy adduct was produced. At 20°, when oxygen was admitted, the spectral change (to the ferric type) showed that oxidation, rather than oxygenation, had occurred. This spectrum was identical to one obtained by addition of N-methyl imidazole to the Fe (III) porphyrin (16a) without prior reduction, and is therefore low-spin six-coordinated ferric.

Reduction could also be carried out with Cr^{II}(acetylacetonate)$_2$ in dry tetrahydrofuran (or benzene _etc._), following the preferred procedure of J. P. Collman [21], or with hydrazine [86] in a variety of solvents (CH_2Cl_2, benzene, _etc._). In the latter case, evaporation of the solvent and addition of deuteriopyridine protected the Fe (II) material from reaction with oxygen,



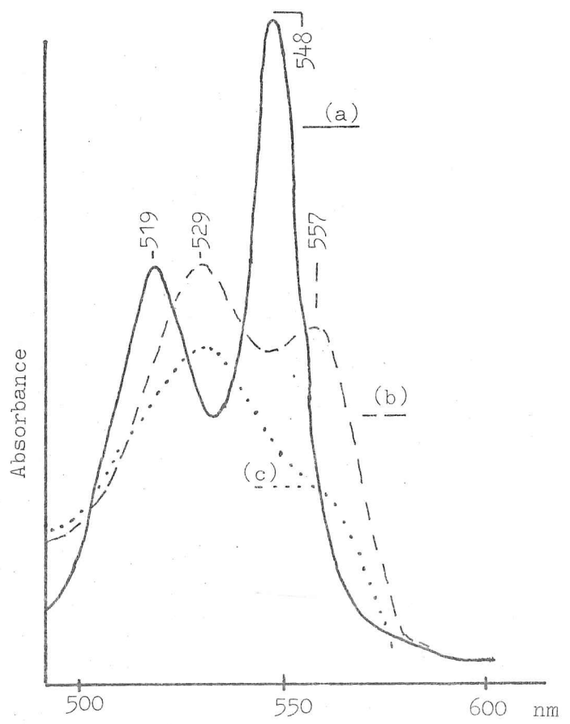
(25)



(26)

(by suppressing the formation of a five-coordinated intermediate to which oxygen might bind [19]) and rendered it sufficiently stable that its n.m.r. spectrum could be obtained (see Chapter 4 and experimental). It was observed, however, that addition of oxygen to a sample in solvents such as tetrahydrofuran, when excess hydrazine was still present, resulted in the bleaching of the solution. The Soret band was greatly diminished, suggesting that the porphyrin had undergone irreversible oxidation, presumably with ring cleavage. Such ring cleavage has previously been studied in coupled oxidation reactions [87]. This complication made hydrazine a poor choice for use with the model compounds when their visible spectra were to be examined, but it remained useful in obtaining n.m.r. samples.

Analogous spectral experiments were then performed with the model compounds (25) and (26). These were the crucial tests of their oxygen-carrying properties. The general features of the spectra produced are shown in Figure 14. The relevant absorption maxima are given in Table 1.



Compound (25) in acetone at 20° (after reduction)

(a) haemochrome with N-methyl imidazole

(b) carbon-monoxy haemochrome

(c) oxygen admitted

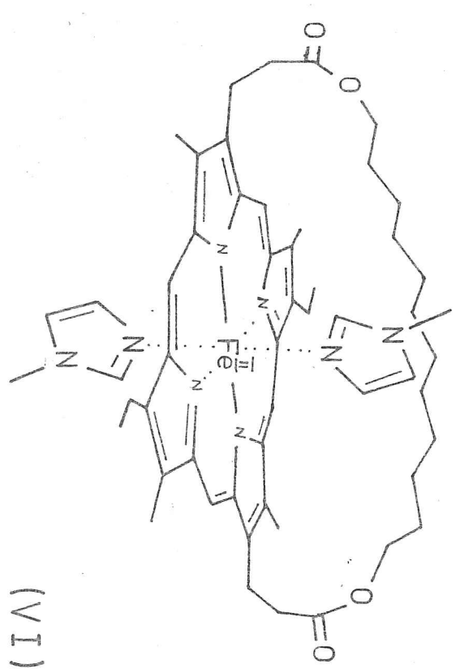
Figure 14

Table 1. Visible Spectral Maxima (nm), for compounds in acetone at 20°.

Compound	Fe(III) Cl^-	Haemochrome Fe(II) plus N-Me Imidazole	Haemochrome plus Carbon Monoxide	Haemochrome plus Oxygen	Fe(III) Cl^- plus N-Me Imidazole
Mesoporphyrin II (16a)	505, 532, 629	518, 547	529, 558	526, 628	526, 628
Acetylenic bridge (25)	505, 532, 630	519, 548	529, 557	526, 630	529, 636
C_{12} Diol bridge (26)	504, 534, 634	524, 554	534, 562	530, 635	528, 630

The surprising conclusion made from these results was that the models behaved exactly as the unbridged (16a). That is, in particular, addition of oxygen to the Fe (II) haemochromes resulted in oxidation to the Fe (III) porphyrin rather than oxygenation, which would probably have been characterised by a spectrum similar to that of Figure 12 (b).

It is not difficult to suggest an explanation for this disturbing result. The visible spectra themselves provide the most significant clue. The ferrous species in the presence of N-methyl imidazole resemble a six-coordinate haemochrome, not a five-coordinate one (compare Figures 10 (a) and 10 (b)). The bridge can be having little effect in preventing the major form in solution from being (for (26)):



(VI)

Oxygen can only react with five-coordinate iron adducts, but if the bridge is having such a small influence on the coordination of the imidazole, then two of these will be present, as shown in Figure 15.

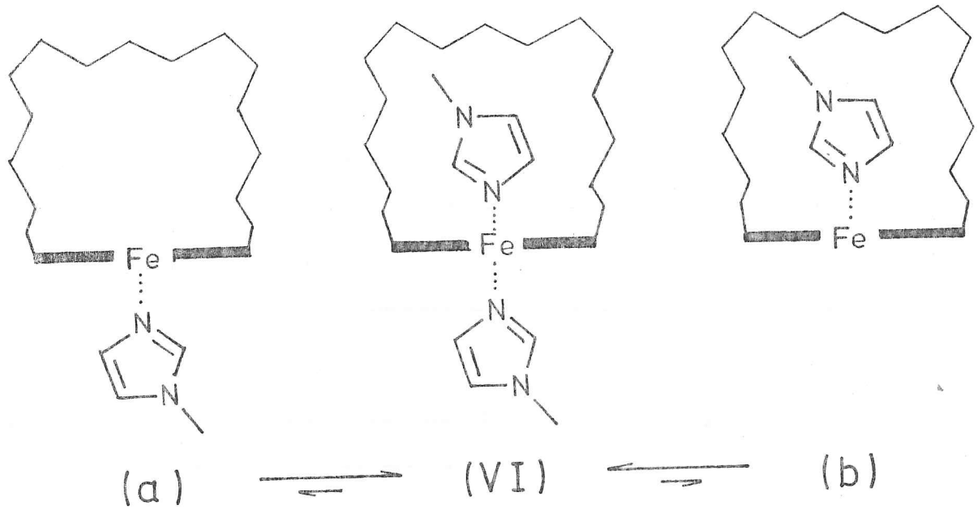


Figure 15

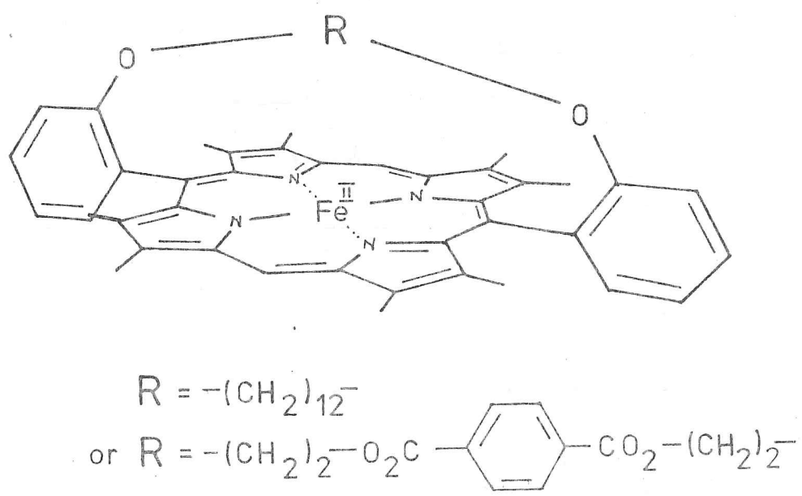
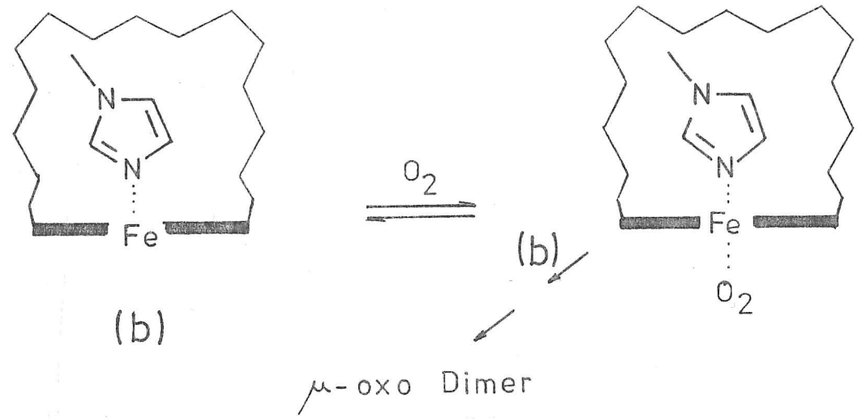


Figure 16

At best, form (a) may be present at higher concentrations than form (b), if the bridge does interfere with the ligand to some extent. However, oxygen attachment to (b) will inevitably result in oxidation:



These steps are clearly rapid at 20°, so the net result will be irreversible oxidation. At that temperature, no change of solvent or reagent for the reduction was of any avail. Rapid oxidation ensued in all cases, although successful attachment of carbon monoxide to the haemochromes confirmed that the spectral interpretations and experimental method were sound.

Since our first studies, two other groups have independently reported similar negative results with singly bridged porphyrins. The work of H. Ogoshi *et al.* is parallel to that described here [80, 88], while J. E. Baldwin's group have prepared the "strapped" porphyrins shown in Figure 16 [89]. These have two substituted meso positions, and are related to the capped porphyrin (which does bind oxygen reversibly at 20° [29]).

Further work designed to circumvent the observed unwanted oxidation is described in Chapter Three.

CHAPTER THREE

a) Introduction to further models

The work described in Chapter Two had laid the foundation for the construction of a wide range of bridged porphyrins, but no reversible room-temperature oxygen carrier had been found. It was therefore essential to determine what future studies could reasonably be undertaken to remedy the shortcomings of the first models. Several lines of development were considered. Each was based on the assumption that it would be necessary to further hinder the approach of an axial base to the iron centre from one side of the macrocycle, without severely interfering with the ability of the oxygen to reach the iron. That is, it was required to encourage coordination of the type (a) in Figure 15 (p. 30) at the expense of type (b), which ideally should be entirely prohibited.

Two simple ideas could be rapidly assessed. One was to increase the size of the axial base and hence reduce coordination as (b) by Van der Waals interactions with the bridge. However, experiments with 4-benzyl pyridine and benzimidazole showed from the visible spectrum that oxidation was still fast, suggesting that no progress could readily be made.

The second possibility was to reduce the length of the bridge and thus move its centre closer to the iron, again increasing steric interactions with the axial ligand on that face. It was decided that such an experiment (with, for example, the iron adducts of (33) or (34)) would be unlikely to succeed, for the approach of the oxygen would also be restricted. Both of these ideas have since been examined in some detail by H. Ogoshi's group, without either leading to reversible binding [80, 88].

Another suggestion was to enlarge the bridge without bringing it closer to the macrocycle. For example, it would have been a relatively simple task to synthesise a diol or diamine with a central benzene ring, or to expand

the bridge in some other way and hence discourage coordination of the axial base. This would have given the model many of the features of the capped porphyrin [29], but since there would still be only two points of attachment, the bridge would be able to move in a lateral direction. As J. E. Baldwin's group had shown in a strapped porphyrin [89] that the bulk provided by a benzene ring in a bridge was insufficient to prevent the unwanted oxidation, this possibility was not pursued.

Instead, a more ambitious project was undertaken. Prompted by the early work of T. G. Traylor's group [42], it was decided to investigate a model having both "bridge" and "tail". It was thought that a molecule such as that of Figure 17 would have many interesting features, and that its synthesis was not beyond the present experimental methodology.

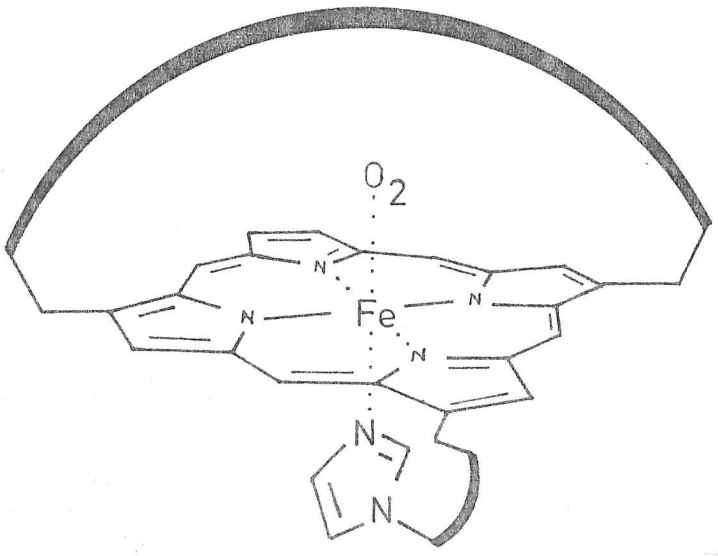
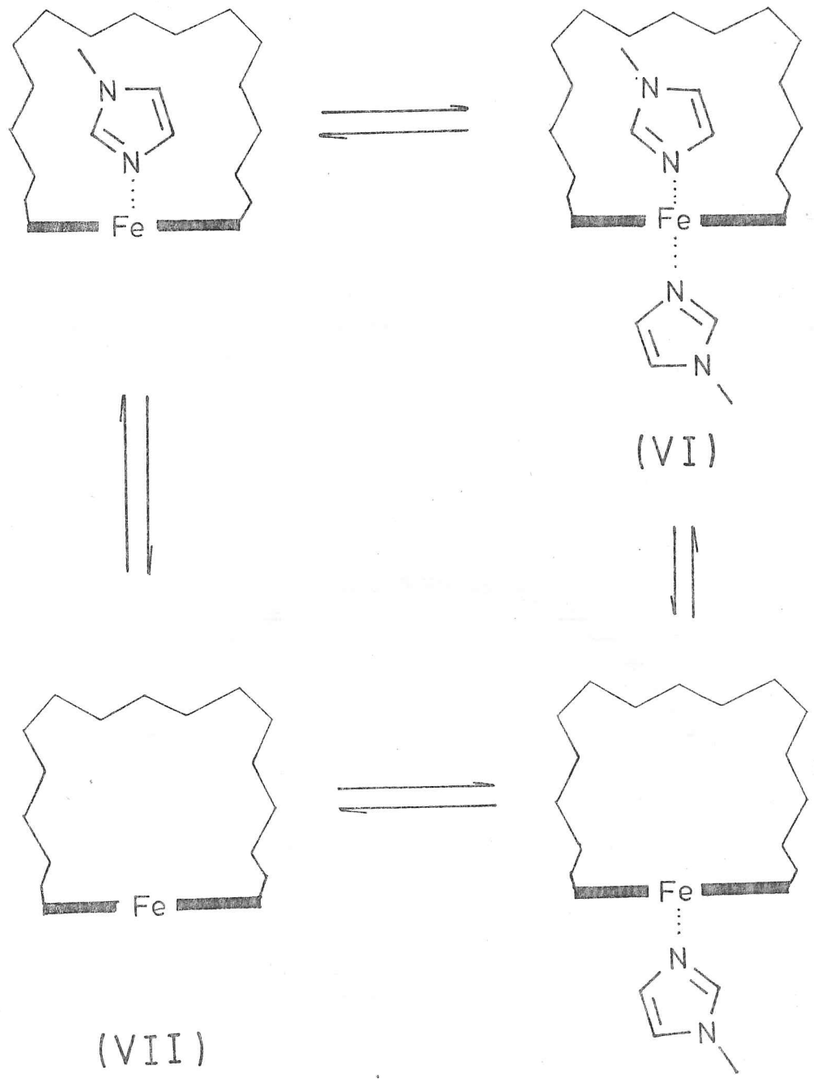


Figure 17

The advantages that might accrue from such a model were apparent. First, it would be a much closer mimic of the active site of myoglobin than any previous model. Secondly, by having only one imidazole ligand per iron before oxygen-binding, the visible spectra ought not to be of the haemochrome type, but should more closely match those of deoxymyoglobin. Thirdly, by



(VI)

(VII)

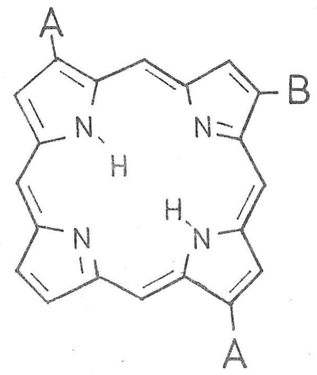
Figure 18

eliminating the need for excess axial ligand in solution during oxygen-binding studies, there should be a reduced tendency for six-coordinate species like (VI) to allow a pathway for the formation of the undesired conformer having the axial base on the same side of the macrocycle as the bridge (Figure 18). The alternative mechanism for ligand transposition, via a four-coordinated intermediate (VII) was expected to be thermodynamically less favourable, on the basis that four-coordination is not observed in the presence of a π-donor ligand.

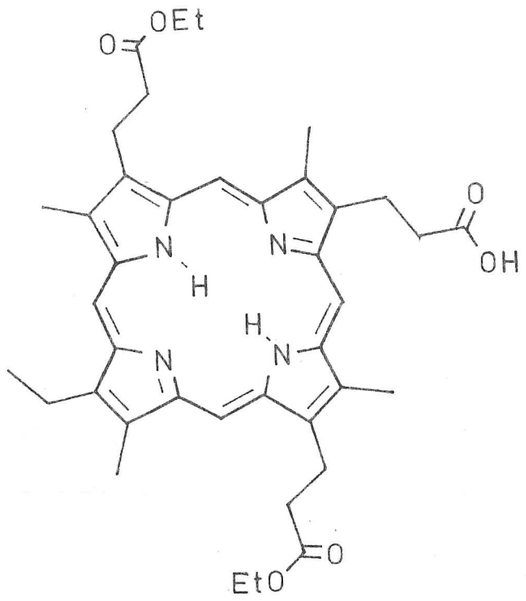
Furthermore, the conformer having the imidazole coordinated alongside the bridge was judged, by inspection of space-filling models, to be less favourable than the alternative with it on the opposite side. In that case, fewer non-bonded interactions were apparent.

b) Synthesis of the Bridge plus Tail model

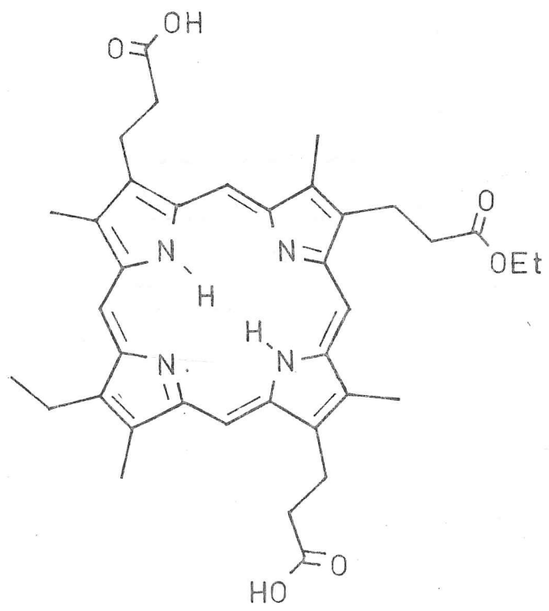
The key to the synthesis of a molecule like that of Figure 17 lay in the construction of a porphyrin such as:



It was essential to be able to differentiate between the points of attachment for the bridge (A) and for the tail (B). For simplicity, the ideal functionalities at (A) and (B) should be propionic acids, for in that case a direct development of the previous synthesis of mesoporphyrin II would be possible.



(35)



(36)

S. G. Hartley, in the Cambridge laboratory, had been examining ways of synthesising porphyrin dipropionates with such a distinction, and it seemed likely from his work that a suitable protecting group for one of the acids would be its ethyl ester [68]. In particular, he had shown that methyl and benzyl esters do not survive the strongly acidic conditions of porphyrin formation from dipyrromethenes. All previous such syntheses contained a reesterification step to restore the esters (usually with methanol and acid) [49].

Two alternatives were therefore available as extensions of our previous synthesis, based on porphyrins (35) and (36). In the case of (35), the plan would be to attach the tail with an amide linkage, hydrolyse the esters under conditions that did not affect the amide, and then attach the bridge. In the case of (36), the steps would be reversed, bridging with a diamine occurring before the tail was connected. It was very difficult to decide between these two alternatives, and both require very similar strategies at the pyrrole level. There is no indication in the porphyrin literature what the physical properties of such a pair of mixed ester / acids might be. The synthesis of the mono acid (35) seemed a marginally more attractive proposition, on the basis that at the porphyrin-forming step it was likely to be more readily separable from byproducts; it would, plausibly, have a greater solubility than the diacid (36). In fact, as will be described, both porphyrins were synthesised and their further chemistry explored.

The route used was a development of the Johnson modification of Fischer's dipyrromethene synthesis, which is applicable to unsymmetrical porphyrins [90]. The general method is to condense two suitably functionalised dipyrromethenes, and then cyclise the resultant a,c-biladiene, as shown in Figure 19. The first step is conventionally catalysed by tin (IV) chloride, and the final cyclisation performed either in dichlorobenzene at reflux or at room temperature in a dimethylsulphoxide / pyridine mixture [49].

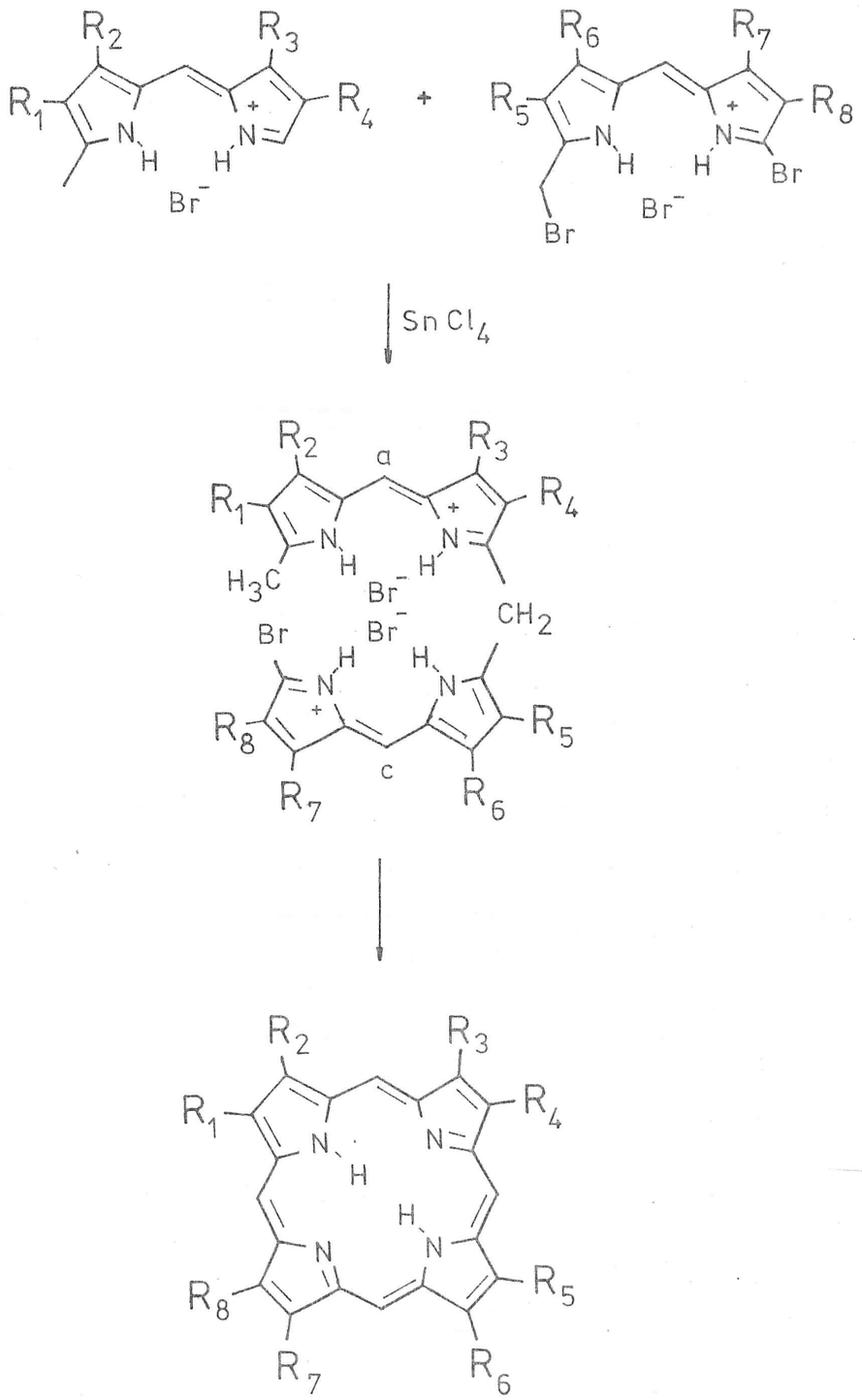
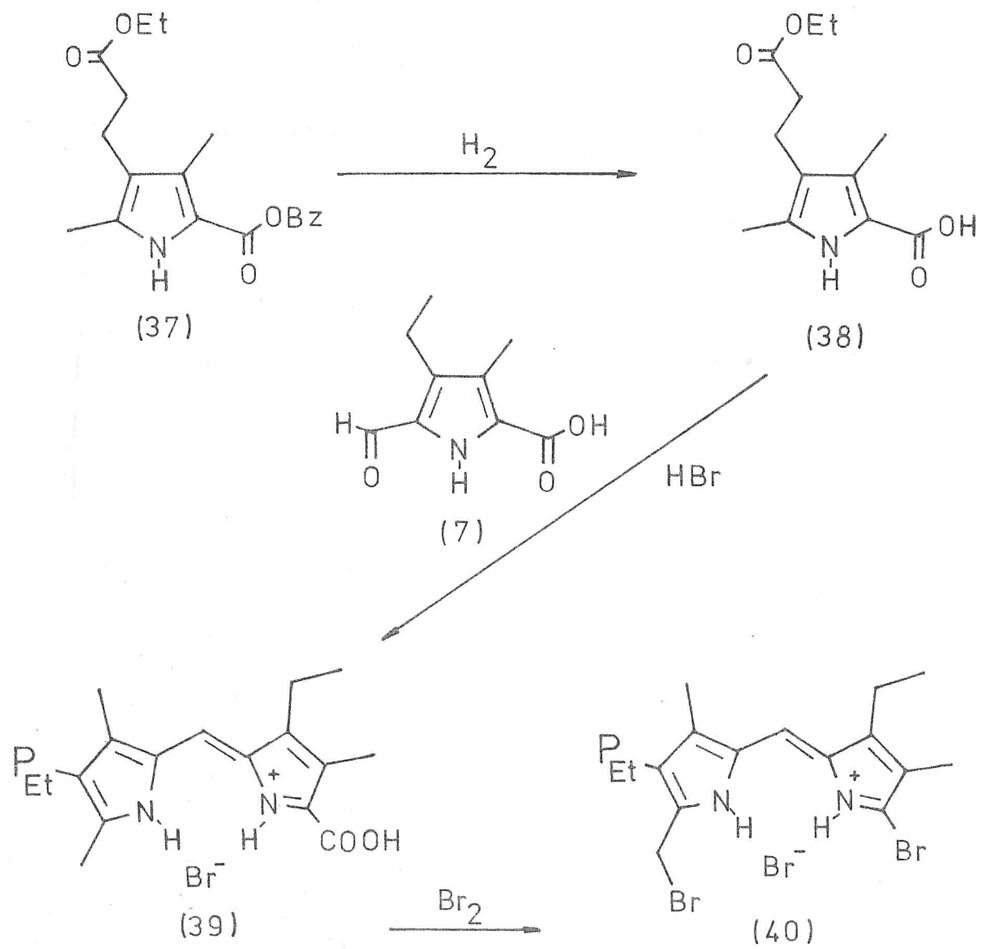


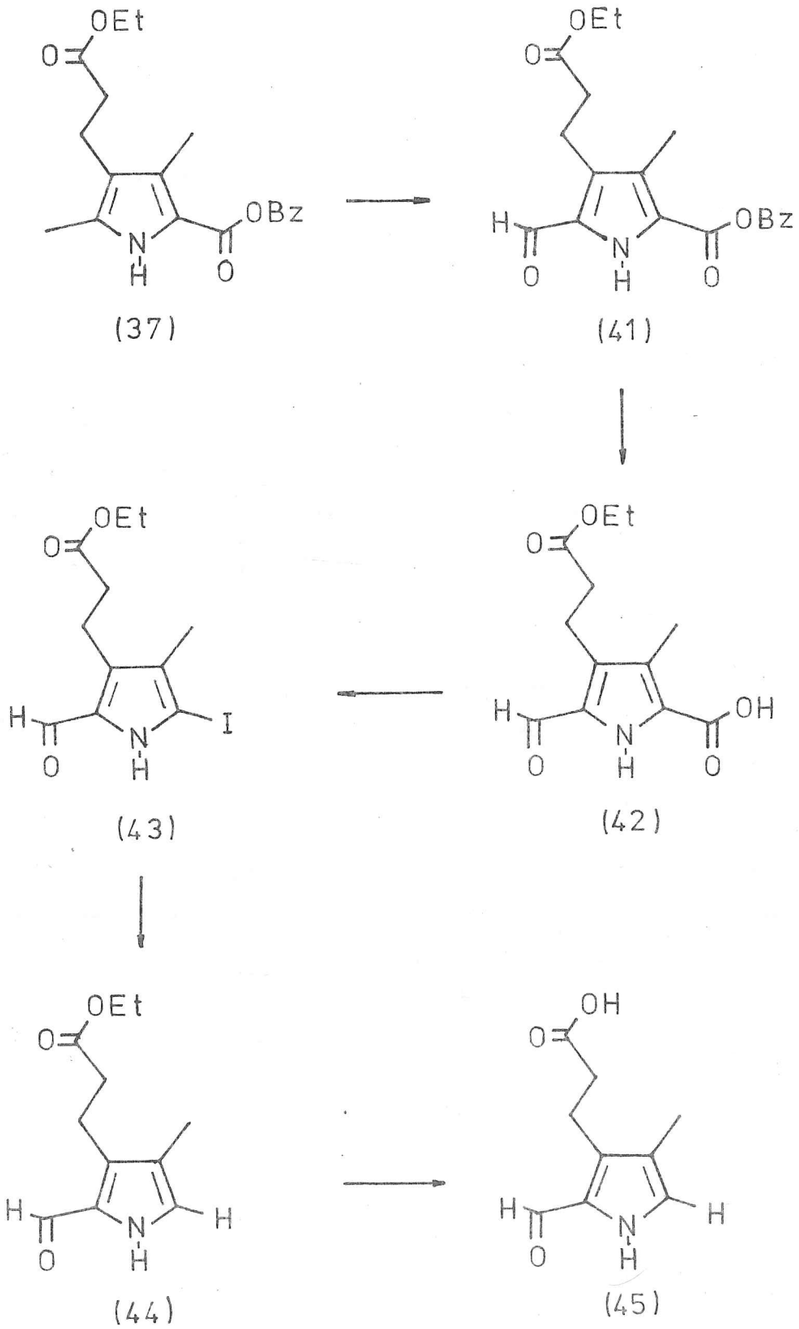
Figure 19

The dibrominated dipyrromethene (40) was readily available, as shown in Scheme 9, and would provide half of the final porphyrin (35).



Scheme 9

This standard sequence started with the Knorr product (37) which was the ethyl ester analogue of the pyrrole (2a) which had been used in the synthesis of mesoporphyrin II. Hydrogenolysis to (38), followed by condensation with the previously prepared aldehyde (7) gave the dipyrromethene (39), which was transformed with excess bromine in boiling dichloroethane to the dibromo



Scheme 10

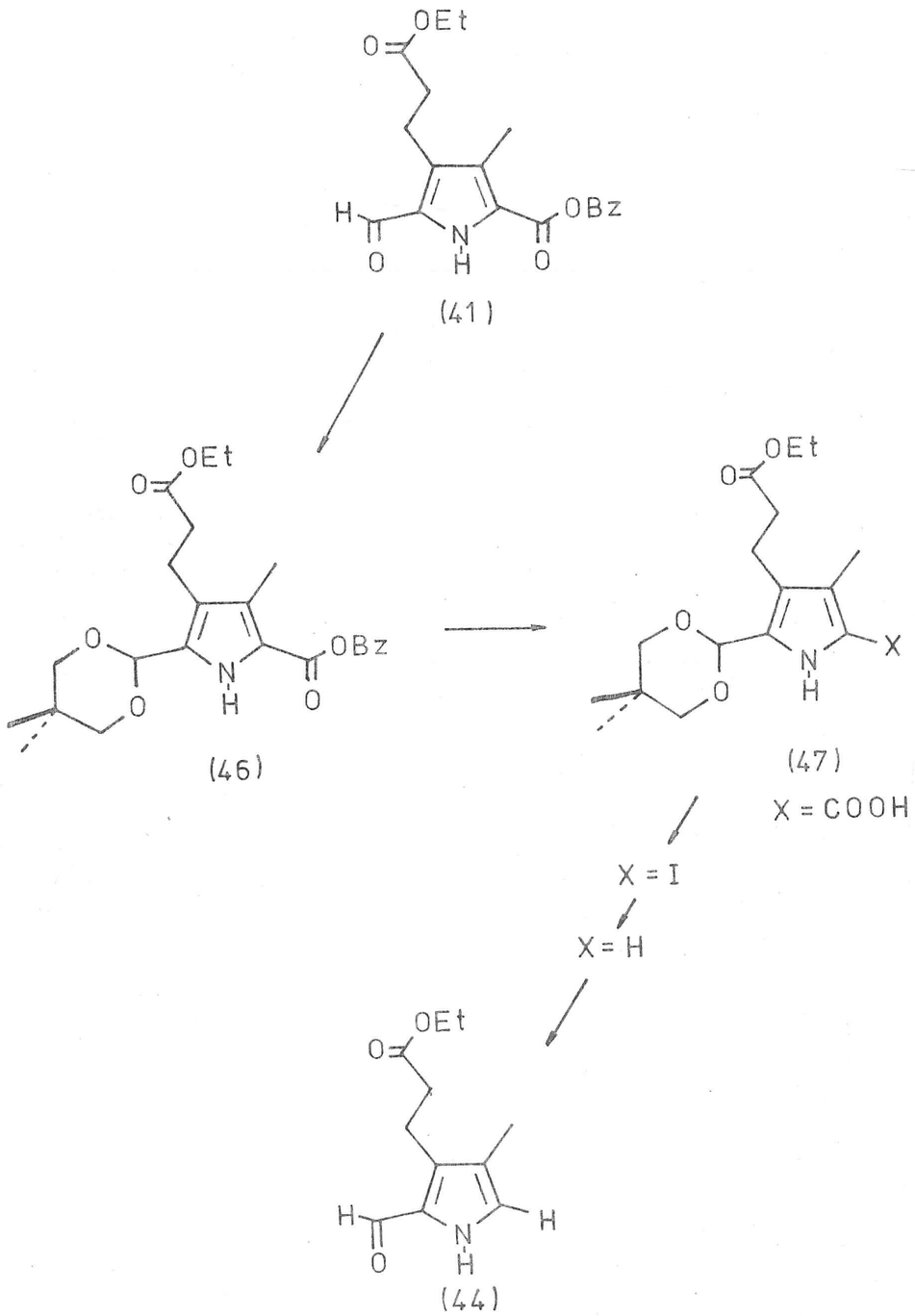
derivative (40). The ethyl ester survived the rather vigorous, but non-aqueous, conditions, and the overall conversion from (37) was 63%. The main loss of material occurred in the bromination, where it was found that the time allowed for reaction was critical, one hour being the optimum.

The second dipyrromethene was required to carry the two distinct propionate groups, and was rather less easy to prepare, because the standard Johnson synthesis demands that the dipyrromethene have a free α-position. The synthesis of the requisite pyrrole (45) is depicted in Scheme 10.

Oxidation of (37) to (41) was again possible with sulphuryl chloride in dichloromethane, as had proved so useful in the synthesis of the aldehyde (7) needed for mesoporphyrin II. The yield in this case was over 75%, and hydrogenolysis to (42) proceeded smoothly provided that the aldehyde (41) was first treated with Raney nickel to remove sulphurous impurities. The α-free pyrrole (45) was then accessible via (43) by a two-phase iodination reaction followed by hydrogenolysis. Although the latter step (to (44)) has been performed in over 80% yield in one case, it was not reliable. Such iodination / hydrogenolysis sequences seem to be very susceptible to minor impurities and can give widely variable yields dependent on the structure of the pyrrole used.

An alternative route to (44) was also investigated. During the course of our work, K. M. Smith reported the novel use of a neopentyl glycol protecting group for pyrrole aldehydes [91], and it was of interest to determine whether this could be used to advantage in the present case. The reactions of Scheme 11 were therefore carried out.

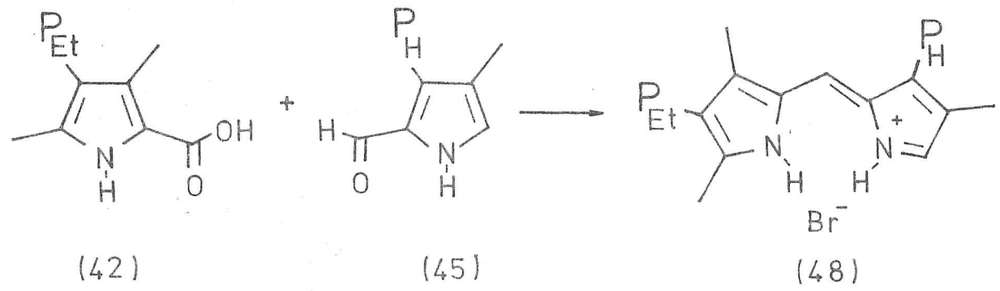
Treatment of the aldehyde (41) (or the mother liquors from the reaction in which it had been formed) with neopentyl glycol and p-toluene sulphonic acid in benzene at reflux gave the protected aldehyde (46). Hydrogenolysis



Scheme 11

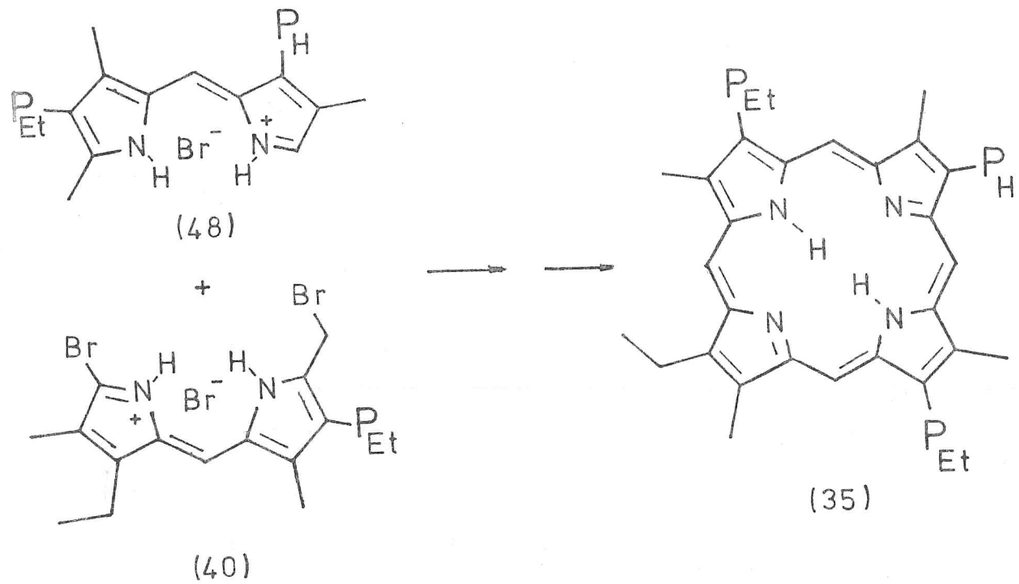
to (47) was straightforward and this nicely crystalline pyrrole was then iodinatively decarboxylated to (44). The intermediate iodo-pyrrole was not isolated, since it appeared to decompose (giving red by-products) on keeping. Instead, the hydrogenolysis was at once carried out on the crude material to provide the α-free pyrrole intermediate. This, too, proved to be highly unstable — it lacks a strongly electron-withdrawing function — and was therefore treated (as suggested by K. M. Smith for deprotection [91]) with trifluoroacetic acid in aqueous ethanol. After this sequence, it was not surprising that the resultant pyrrole (44) could only be purified by chromatography, and was then obtained in poor yield; less than 5% overall from (46). Although the yield was not optimised, it was clear that the preparation described above, without the use of a protecting group, was superior.

Alkaline hydrolysis of (44) gave (45), and this pyrrole was condensed with (42) to give the second dipyrromethene required for the synthesis of the porphyrin:

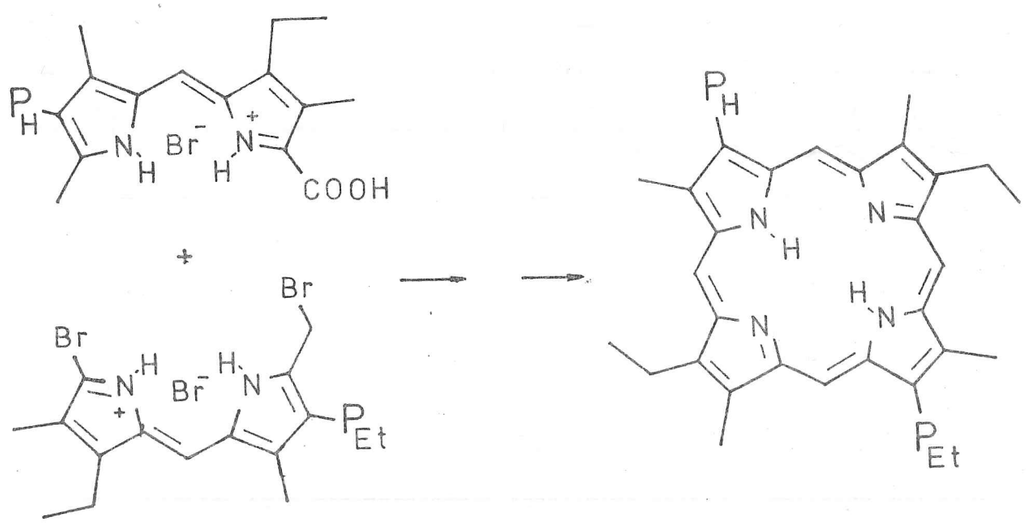


(42) (45) (48)

The dipyrromethene (48) so produced was then coupled with the dipyrromethene (40), as shown in Scheme 12, to complete the synthesis of the porphyrin (35). This condensation, in dichloromethane containing tin (IV) chloride, is usually worked up in such a way as to keep any carboxylic acids fully esterified [90]. In this case, it was clearly important to avoid esterification



Scheme 12

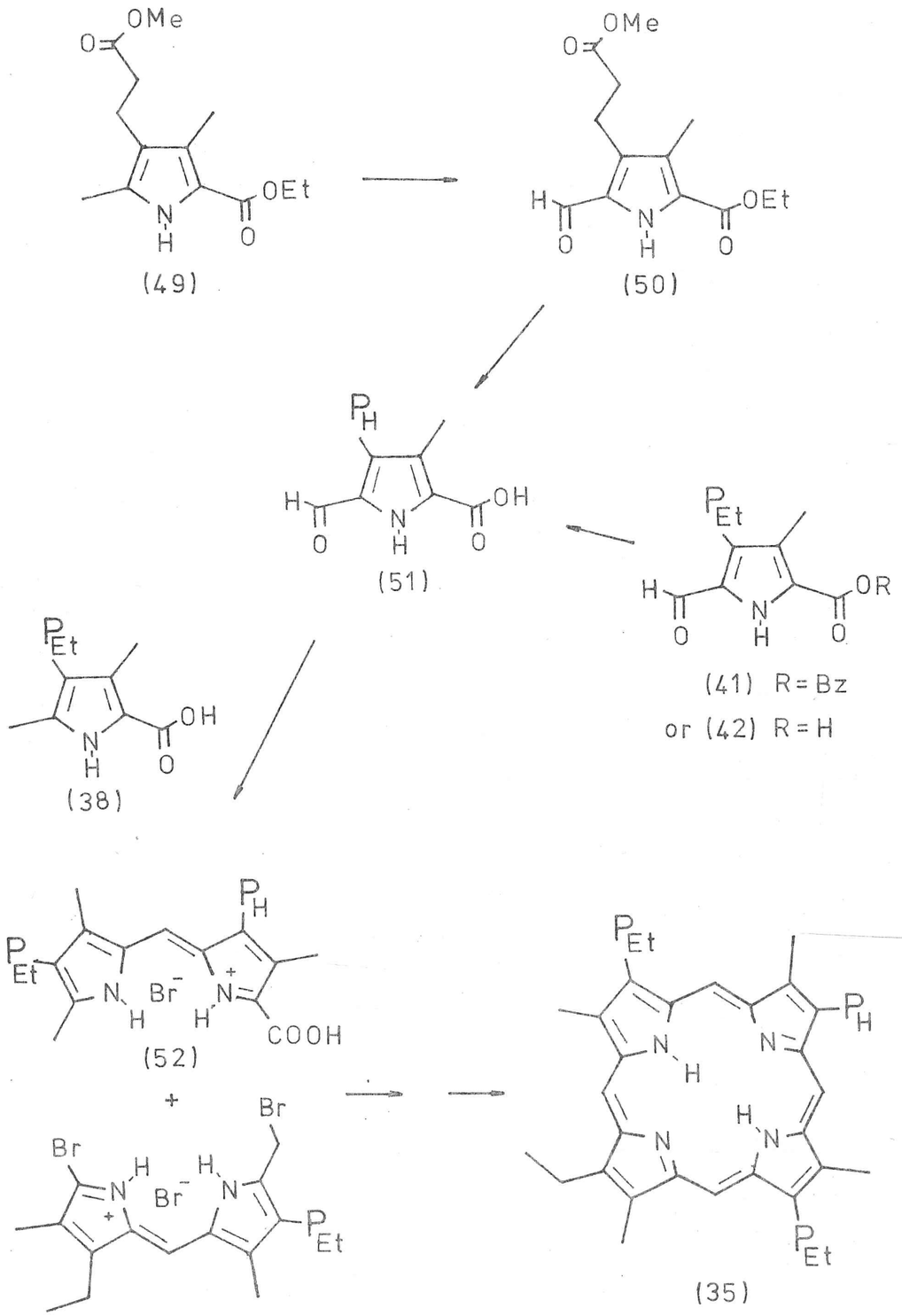


Scheme 13

of the single free acid (or, indeed, to allow hydrolysis of the esters already present), so a method devised by S. G. Hartley was employed [68]. The biladiene was washed free of tin salts while held in solution in dichloromethane. Since the biladiene is moderately soluble in water, a high concentration of ammonium bromide was maintained in the aqueous layer, which also contained 5% hydrobromic acid. Under those conditions, the biladiene was largely present in the organic phase. It was not purified at this stage, but cyclised at once to the porphyrin (35), in dimethylsulphoxide and pyridine at 20°. The porphyrin was successfully separated from the inevitable tarry by-products by column chromatography, and was obtained in about 26% yield from the dipyrromethenes. Small amounts of other porphyrins were observed in the reaction mixture by thin-layer chromatography, but they were not fully characterised. They were presumably the result of hydrolysis and / or transesterification.

A significant improvement in the overall yield would be possible if there were no requirement for an α-free dipyrromethene in the biladiene-forming reaction. By analogy with the formation of mesoporphyrin II and similar centrosymmetric porphyrins from the self-condensation of an α-COOH dipyrromethene, it seemed likely that such a dipyrromethene might be induced to react with the dibromodipyrromethene (40). S. G. Hartley carried out the conversion shown in Scheme 13 by that method, and found that an important requirement was to leave the reaction mixture for about 15 days at room temperature to allow full conversion to the biladiene (as judged by the visible spectra) [68, 82].

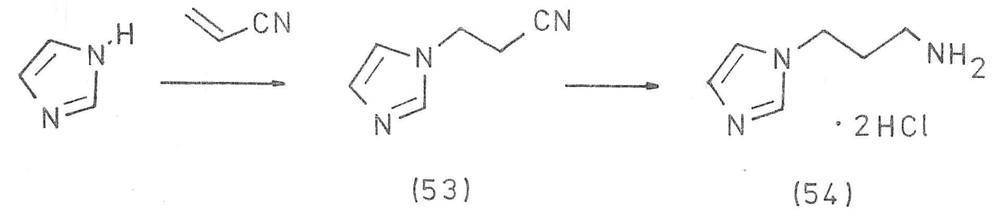
This was in contrast to the reaction of (48) with (40) under the same conditions, which was complete in less than an hour! In order to apply the method to the synthesis of (35), it was necessary to prepare the dipyrromethene (52), as is shown in Scheme 14.



Scheme 14

Although the diacid (51) was available from the hydrolysis of (41) or (42), and this provided an early source, a cheaper supply was forthcoming from the Knorr product (49), having simpler ester substituents than (37). Oxidation to (50) and hydrolysis gave (51) in 53% overall yield. Coupling with the previously prepared pyrrole (38) gave the new dipyrromethene (52) in 89% yield. Cyclisation with (40) to afford porphyrin was then possible in 14% yield.

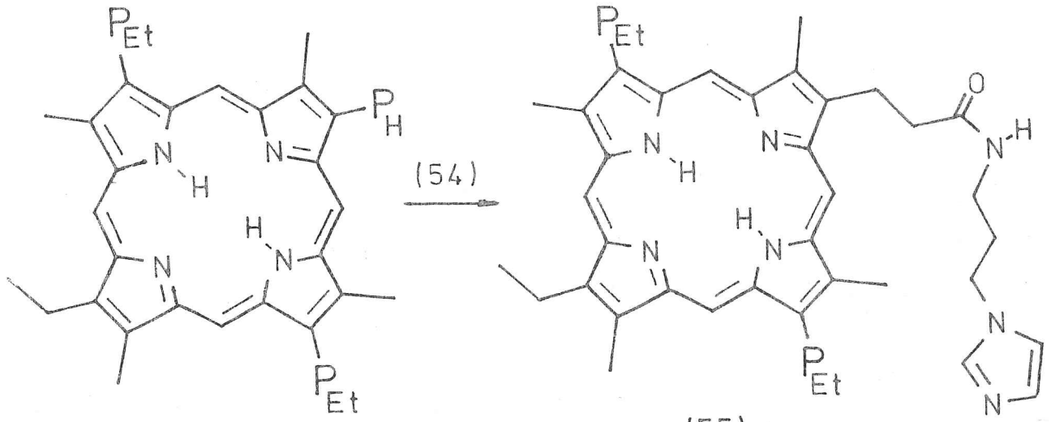
Following the plan for the synthesis of the bridge and the tail, the reactions of Scheme 15 had next to be applied. The amine, 1-(3-aminopropyl)-imidazole (54), which has been used before to provide a tailed porphyrin [35], is available following a literature method [92] from imidazole:



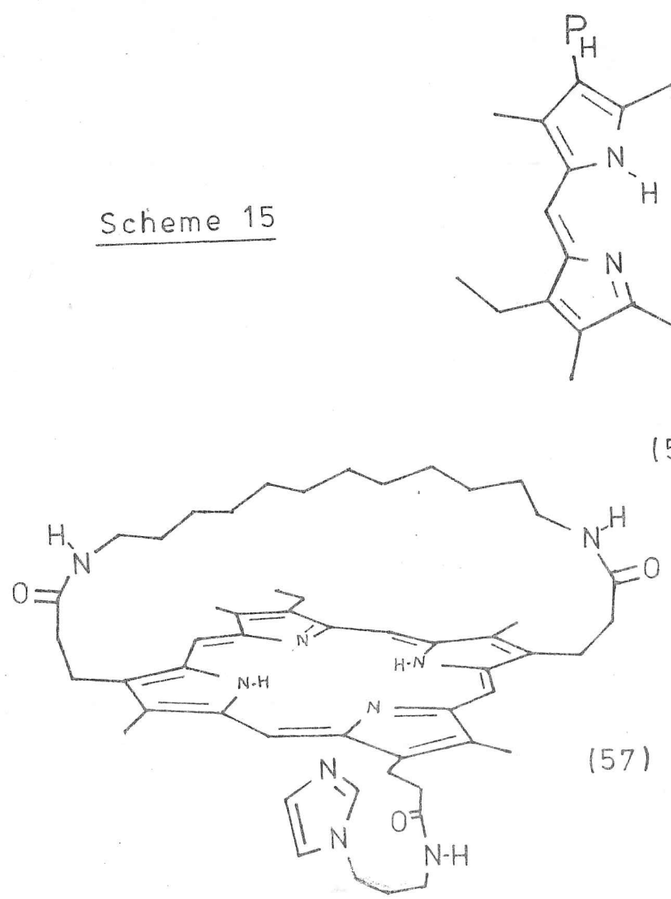
After Michael addition of imidazole to acrylonitrile, the resultant nitrile (53) was reduced with hydrogen and Raney nickel to give (54), isolated as its dihydrochloride.

When the acid chloride of the porphyrin (35) (prepared from it by treatment with oxalyl chloride) was treated with the amine free base, the amide diester (55) resulted.

The important step of hydrolysing the esters to their free acids, without interfering with the amide, was then investigated. Treatment of (55) in 6 N hydrochloric acid at 20° readily completed the transformation, but the



Scheme 15

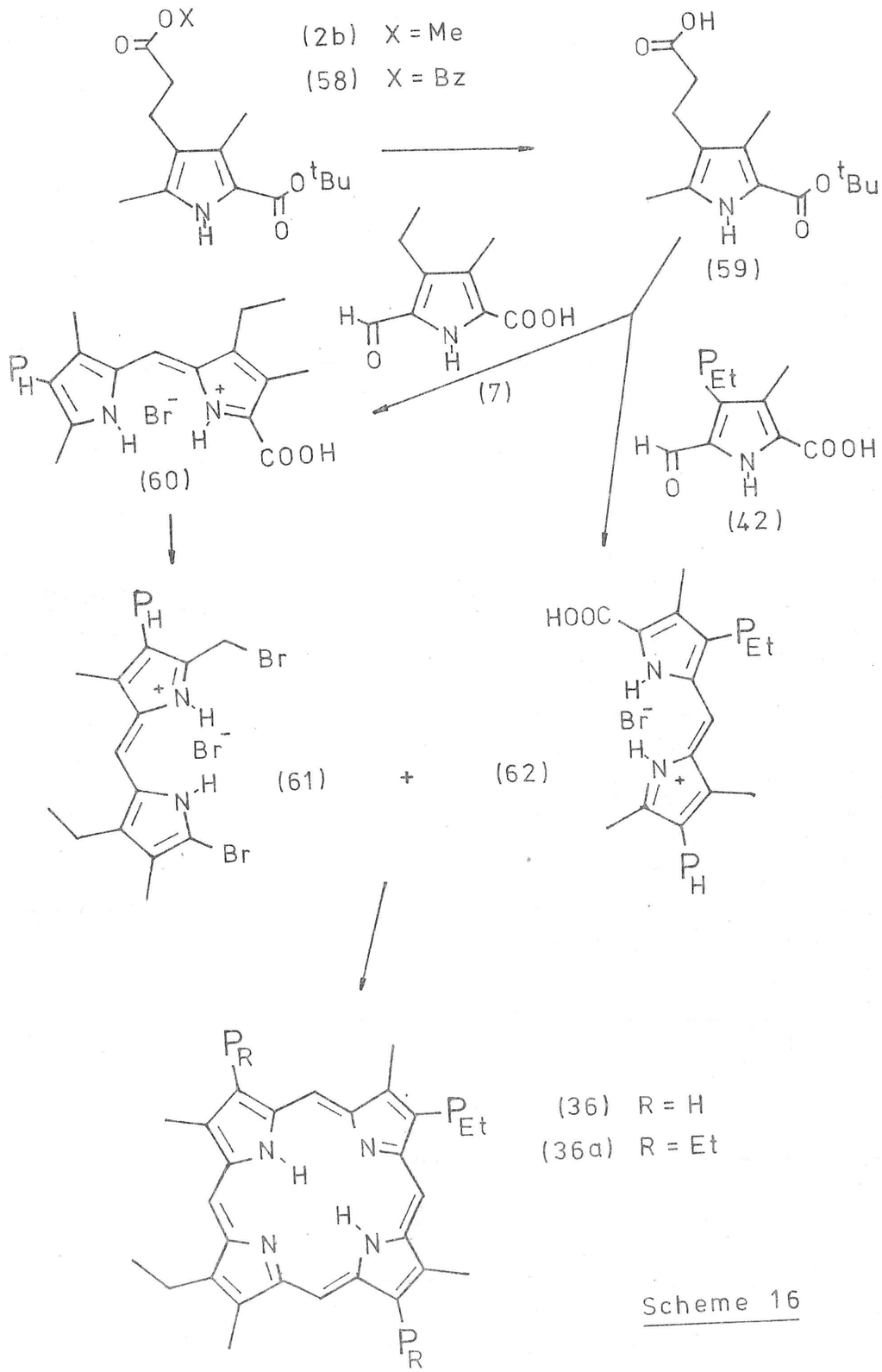


resultant free acid (56) was very difficult to handle. It proved to be totally insoluble in most organic solvents, except water-miscible ones. The best purification method found was to precipitate it from aqueous acid with base, but it was never obtained analytically pure; although its structure is not in doubt as the n.m.r. was quite characteristic.

The final step of this long synthesis of the desired system (57) was even more frustrating. It was planned to form the diacid chloride of (56) with oxalyl chloride and to treat it with the long-chain diamine, as had previously been so successful. However, no solvent could be found for (56) that was compatible with oxalyl chloride. While the compound was soluble in methanol, dimethyl formamide, dimethyl sulphoxide, N-methyl pyrrolidone, and pyridine, each of these reacts violently with oxalyl chloride. In contrast, solvents which did not decompose the reagent were quite unable to dissolve the substrate and, even in chloroform at reflux, it would not react with oxalyl chloride. It had been noted that mesoporphyrin II, which is almost insoluble in dichloromethane or chloroform, does react readily at room temperature, presumably because a little dissolves and is rapidly transformed to the freely soluble bis acid chloride.

As a final expedient, the effect of neat thionyl chloride on the diacid (56) was investigated, as this had been perfectly acceptible for the conversion of mesoporphyrin II to its bis acid chloride. However, although (56) did indeed dissolve, a "sting in the tail" was then revealed! The solution rapidly became green in colour and when, after evaporation of excess reagent, it was quenched with an alcohol, no porphyrinic material could be isolated. It is conjectured that the basicity of the imidazole is sufficient to render the acid chloride unstable, either by ketene formation (with subsequent polymerisation) or nucleophilic attack by the porphyrin itself, after activation as an acid imidazole derivative.

However, an alternative route to (57), outlined below, was soon successful, so further experimentation based on the above intermediates was



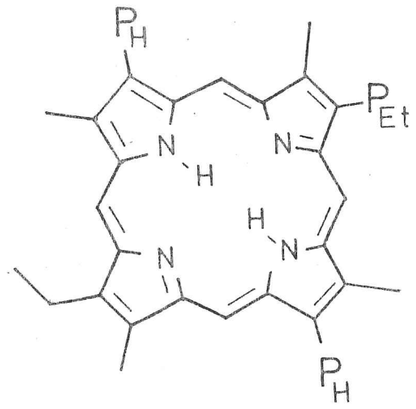
Scheme 16

unnecessary. Possible measures considered included reacting the diester (55) directly with the diamine at high temperature under the catalysis of 2-pyridone, following methods which produce amides directly from esters [93]. The experiment was performed in a sealed tube with mesoporphyrin II dimethyl ester (16), and did give a tiny yield of the corresponding bridged porphyrin (31), but it was hardly a practical proposition for a synthesis intended to yield several hundred milligrams.

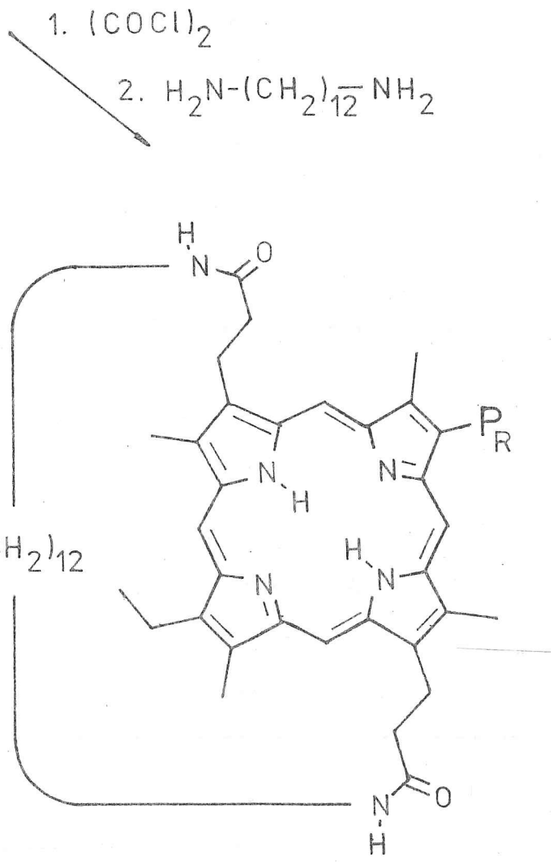
The alternative approach was the one mentioned at the beginning of the chapter, namely to attach the bridge first and then the tail. The required porphyrin (36) was synthesised by an extention of the methodology already developed (Scheme 16).

Transesterification of (2b) to the diester (58) was accomplished in hot benzyl alcohol containing sodium benzyloxide [68]. Hydrogenolysis then gave the pyrrole-β-propionic acid (59), which was condensed with the aldehyde (7) to provide the dipyrromethene (60) in 80% yield from (2b). Bromination to (61) was carried out with excess bromine in dichloroethane at reflux, but owing to the low solubility of (60), it took longer (3 hours) than had been needed in the earlier preparation of a dibromodipyrromethene. This product was still contaminated with some monobromo material, as judged by the n.m.r., but was quite satisfactory for the further reaction with the dipyrromethene (62); itself readily prepared from the pyrroles (42) and (59) in 94% yield. The dipyrromethene (62), rather than an α-free dipyrromethene, was the chosen nucleophile for reaction with (61), following the use of such a species in the preceding porphyrin synthesis. On this occasion, the combination of dipyrromethenes gave a 32% yield of the porphyrin (36).

Fortunately, it was quite feasible to purify (36) by column chromatography, as it has a much greater solubility in solvents like chloroform than might be anticipated by comparison with the other diacids studied, either mesoporphyrin II, or the notorious tail diacid (56). It was striking that

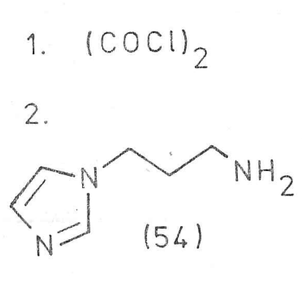


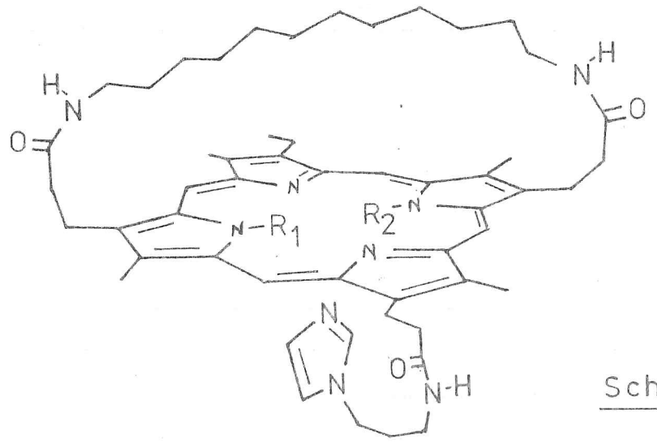
(36)



(63) R = Et

(64) R = H





(57) $R_1 = R_2 = H$

(65) $R_1 - R_2 = -Fe^{III}(Cl)-$

Scheme 17

small quantities of an alcohol like methanol increases markedly the solubility of (36) in chlorinated solvents, although the pure compound is not appreciably soluble in methanol. This effect has also been found with other porphyrins, especially free acids.

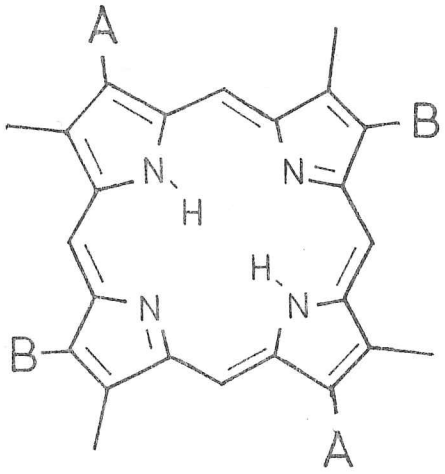
To strengthen the structural assignments in this porphyrin, the triester (36a) was prepared from it and compared with the triester produced by esterification of (35). As expected, these compounds were identical in every respect.

The synthesis of the model compound (65) was then completed as shown in Scheme 17. Attachment of the bridge was effected in the way developed for mesoporphyrin II, by the direct coupling of dodecane-1,12-diamine and the diacid chloride of the porphyrin. The yield of (63) was 38% on a one gram scale. Hydrolysis of the ester was complete in 9N hydrochloric acid after 3 hours at 20°. No hydrolysis of the amides was apparent, and the free acid (64) was obtained in 83% yield. Attachment of the tail proved to be less successful on the largest scale attempted (ca. 300 mg) than it had been on smaller scales. The acid chloride was again formed in dichloromethane by treatment with oxalyl chloride, and the resultant material was immediately treated with excess of the tail amine (54) as its free base. Chromatography was required to separate the product (57) from other material, including excess amine and polymeric porphyrinic by-products. The yield was only 35%, but in order to avoid tedious repetition of the earlier stages, this amount was accepted as sufficient for the further experiments. It is felt that an improved yield would be achieved in any repetition of this step.

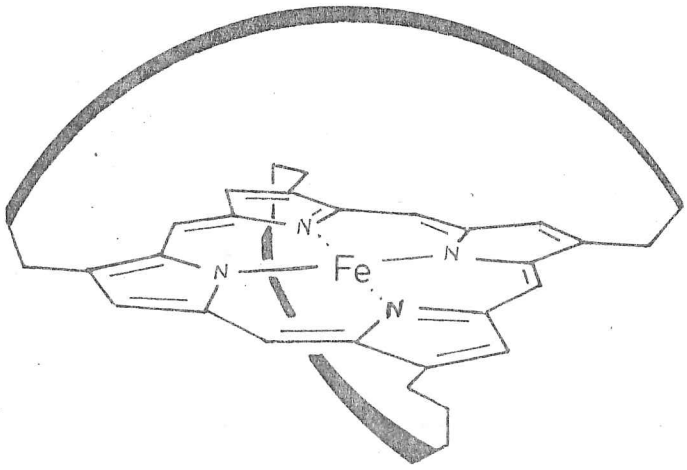
Iron insertion was readily accomplished, so the desired model compound (65) was at last available for model studies. These are described in Section (d) of this chapter.

c) Synthesis of the Double Bridge model

While work was in progress on the bridge plus tail model (65), it was realised that an alternative method of preventing the problems posed by the earlier work was accessible. A straightforward conversion of some of the pyrroles available would allow the synthesis of a porphyrin like (VIII) below, and hence of a model such as (IX), with a pair of bridges connected to the macrocycle:



(VIII)



(IX)

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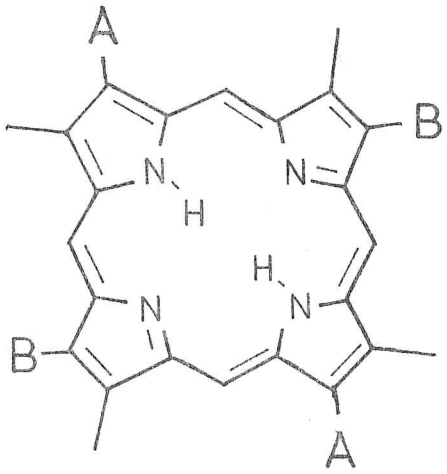
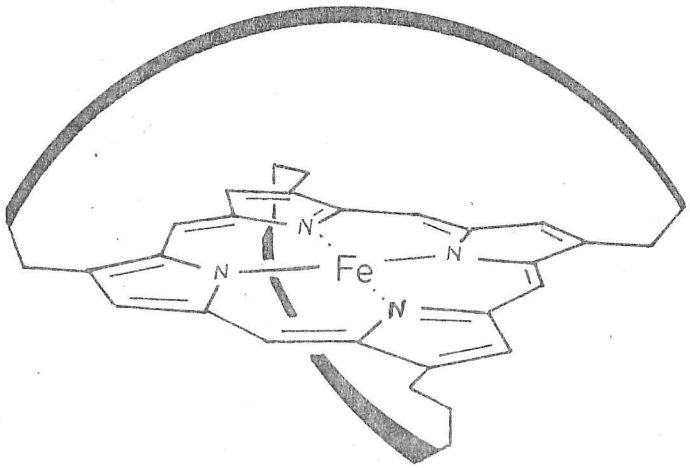
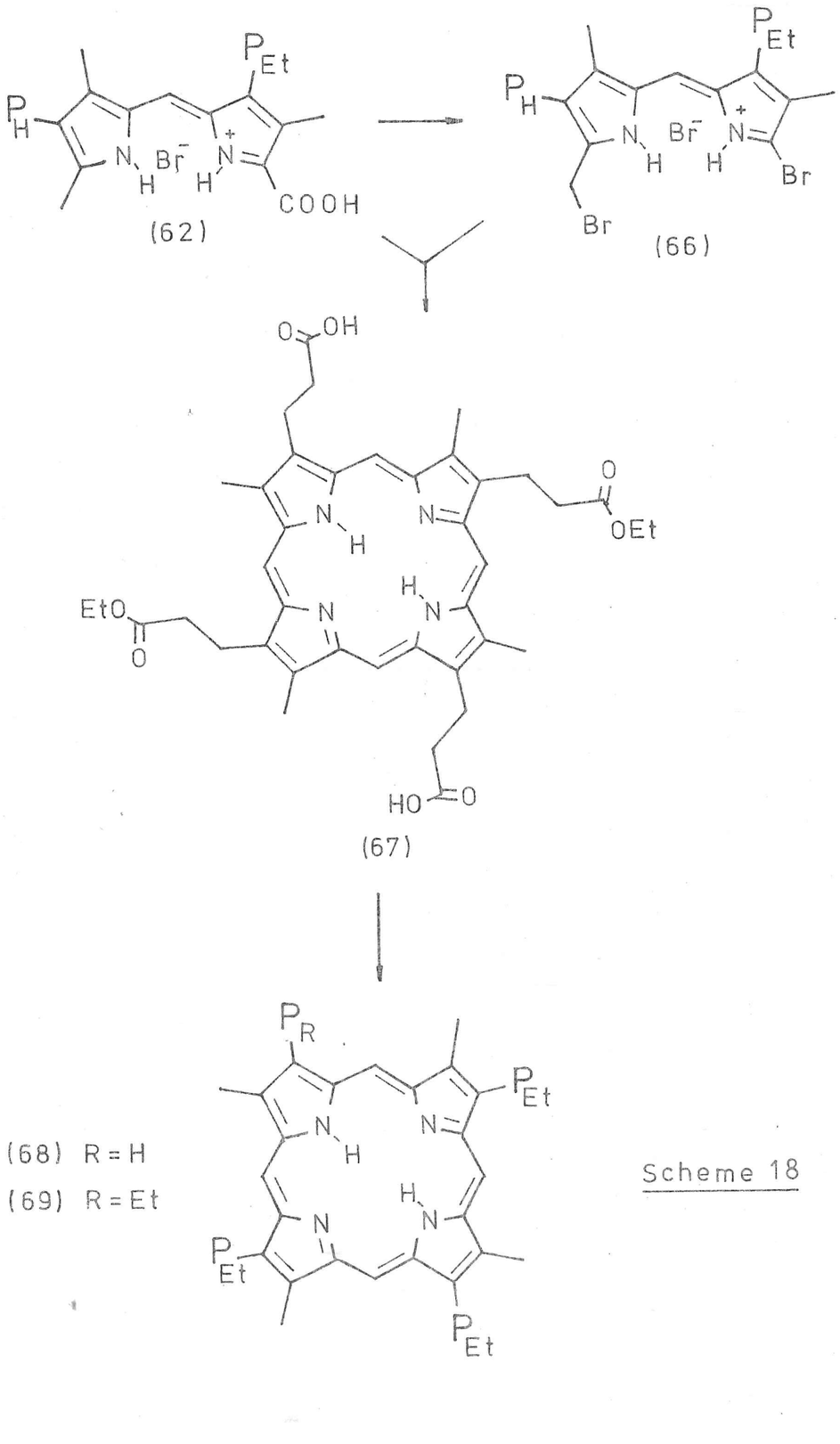


(VIII)



(IX)



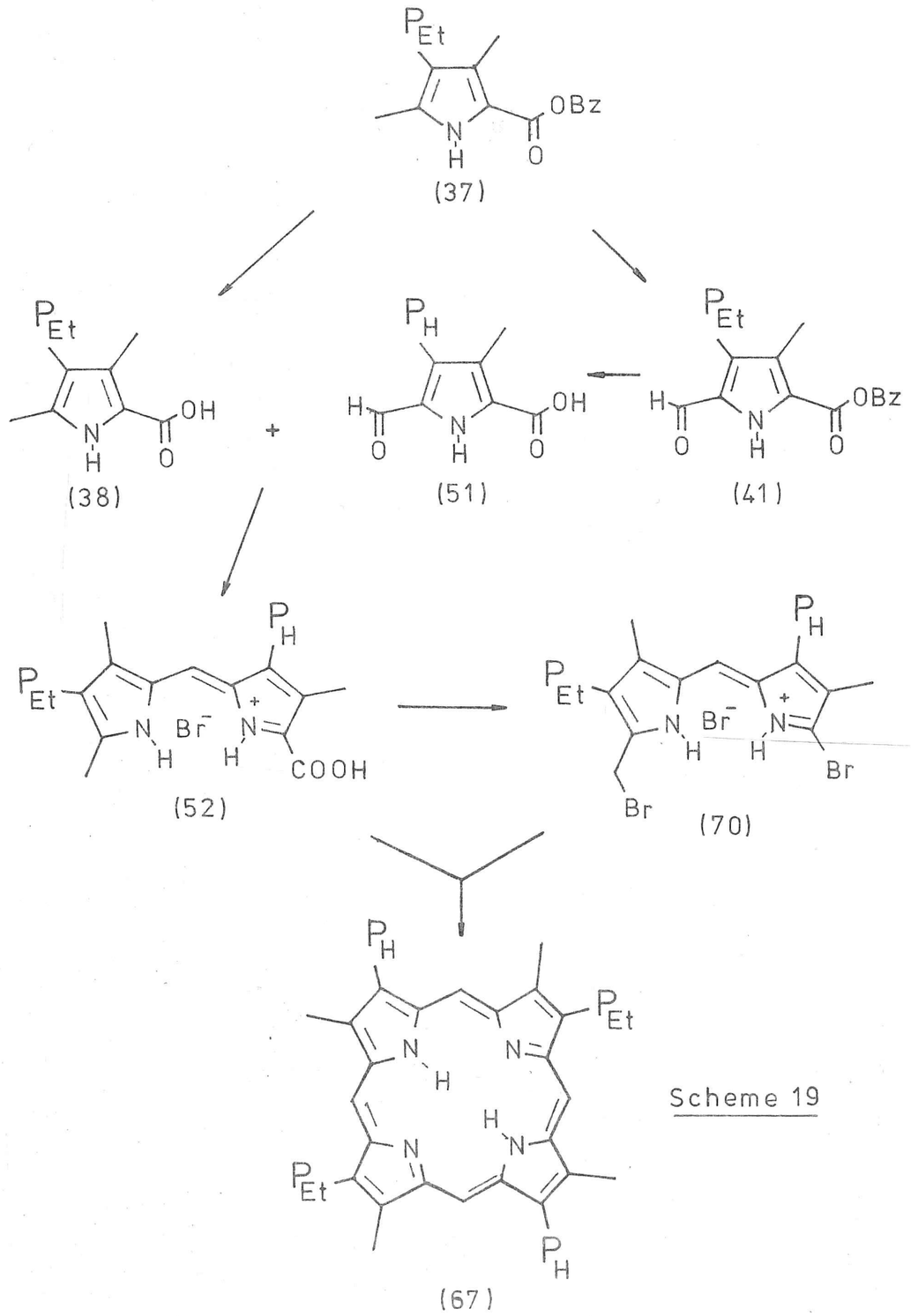
Scheme 18

Such a molecule was very attractive as a potential oxygen-carrier. It was hoped that having double bridges, one over each face of the porphyrin, would prevent aggregation to form dimers. Even if there were excess axial ligand present in solution, and hence both five and six-coordinate species, in no case should two porphyrins be able to come sufficiently close to form a μ-oxo dimer.

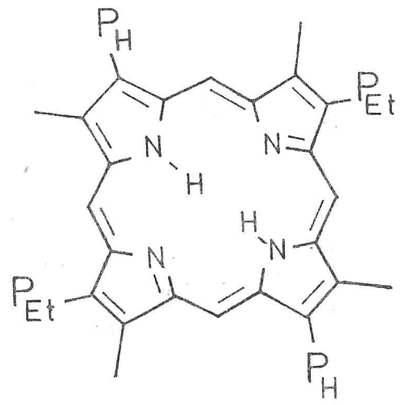
The porphyrin synthesis used is depicted in Scheme 18. The dipyrromethene (62) was brominated in 1,2-dichloroethane at reflux to give the dibromodipyrromethene (66) in about 80% yield. This could then be coupled with more of the unbrominated (62), by the same method as had been used before: stirring at 20° in dichloromethane containing tin (IV) chloride. The porphyrin (67) was subsequently obtained in over 26% yield. Spectral data were in full accord with the proposed structure, but analytical samples repeatedly crystallised from dichloromethane / ethanol (or chloroform / ether) did not give a satisfactory combustion analysis. The carbon content found in the best sample prepared was 66.33%, while that required in theory is 67.6%. For that reason, the tetra-ethyl ester (69) was also prepared, as this is a known compound, a coproporphyrin I derivative [52]. It was formed by the esterification of (67), and did give entirely consistent analytical data.

The triester (68) was a by-product of the porphyrin-forming reaction, and was characterised in order to compare its n.m.r. spectrum with those of (67) and (69) (see Chapter 4).

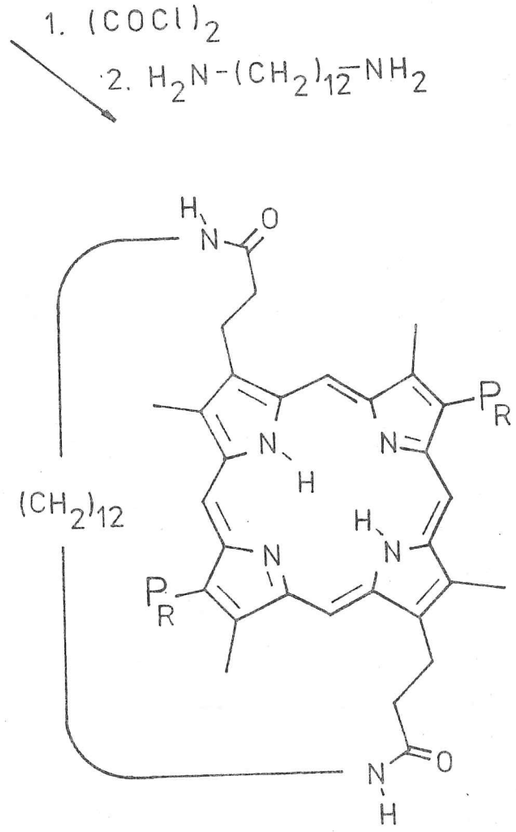
An alternative synthesis of (67) would have been equally practicable from the pyrroles that were available, and is shown in Scheme 19. These reactions were carried out for comparison with the previous scheme, and found to have no particular advantage, other than the aesthetic one of having all intermediates accessible from a single Knorr product (37).


P_Et
OBz
N H
O
(37)
P_Et
OH
N H
O
(38)
+
P_H
H
OH
N H
O
O
(51)
P_Et
H
OBz
N H
O
O
(41)
P_H
P_Et
N H Br⁻ N H
COOH
(52)
P_H
P_Et
N H Br⁻ N H
Br
Br
(70)
P_H
P_Et
N H
N
H N
N
P_Et
P_H
(67)

Scheme 19



(67)

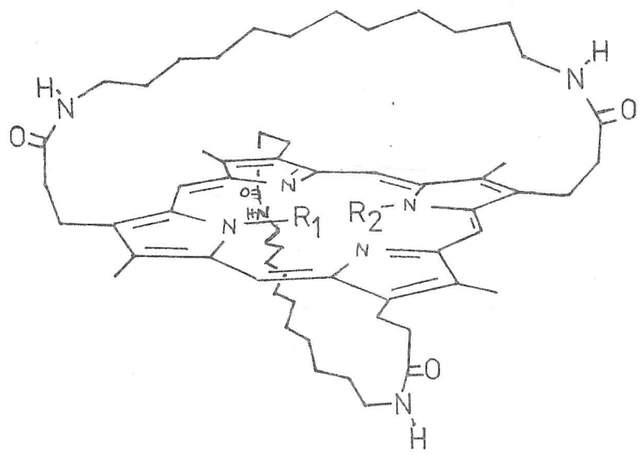


(71) R=Et

(72) R=H

1. $(COCl)_2$

2. $H_2N-(CH_2)_{12}-NH_2$



(73) $R_1 = R_2 = H$

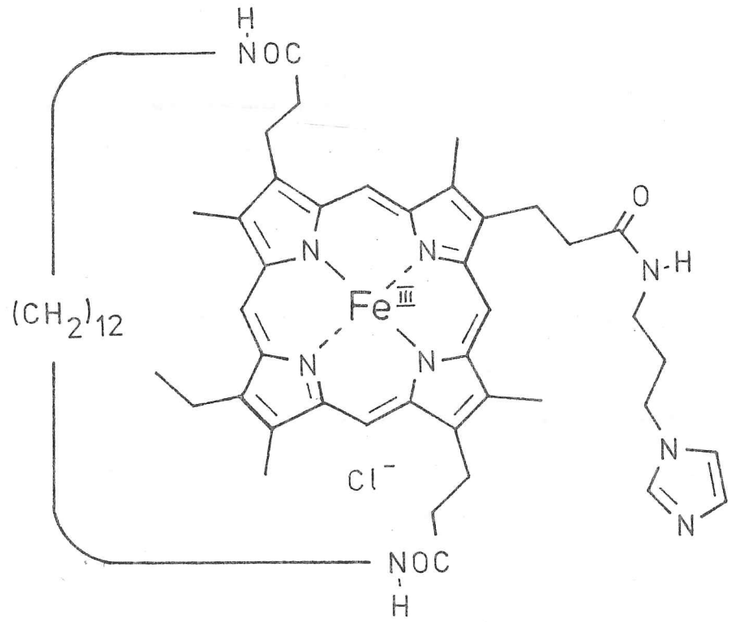
(74) $R_1 - R_2 = -Fe^{III}(Cl)-$

Scheme 20

The subsequent conversions of the bis acid, bis ester porphyrin (67) followed the plan of the synthesis of the bridge plus tail model (65), and are shown in Scheme 20. The first bridge was attached to the bis acid chloride of (67), giving (71) in 33.5% yield. Hydrolysis to (72) proceeded in 87.5% yield, and the second bridge could then be attached by the same method. This reaction, which was only carried out once on a relatively large scale (600 mg of (72)), gave a disappointingly low yield of (73) – 11.5%. It is possible that the presence of one bridge interferes with the formation of the second, but alternative explanations for the less successful reaction cannot be ruled out. For example, some water may inadvertently have entered the system.

There was no evidence for the formation of the bridged compound having both bridges on the same side of the macrocycle. Such a molecule would lack the $\bar{4}$ symmetry axis of (73), and this would be revealed in the n.m.r. spectrum. The bridges must then of necessity lie at different heights over the plane of the porphyrin and the consequent chemical shifts would not resemble those of singly bridged models. In fact, the product obtained had an n.m.r. spectrum fully consistent with its symmetry, having a singlet for the meso protons and for the four aromatic methyl groups: the pattern of signals for the bridge protons was identical to that of the singly bridged model (see Chapter 4).

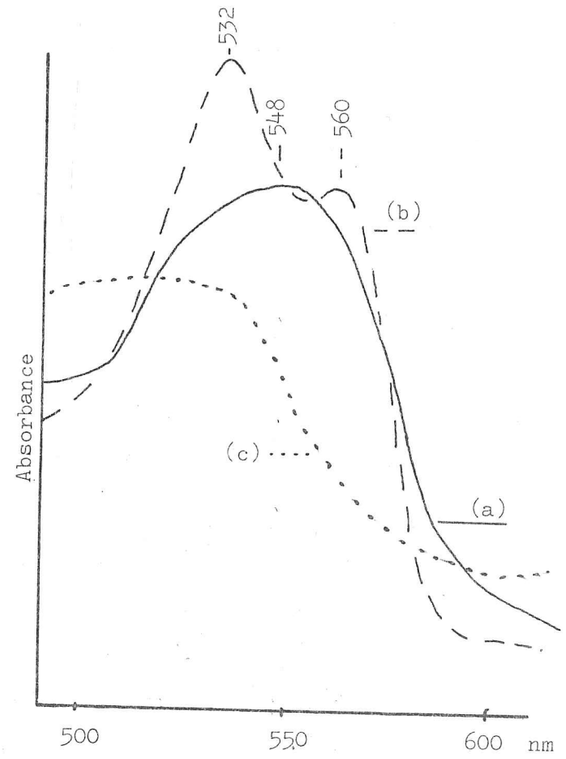
Iron insertion into (73) gave (74), the last model system made in the present series. Analytical and spectroscopic data matched the assigned structure, following isolation as the Fe (III) chloride salt. Combustion analysis confirmed the presence of one molecule of water, which is undoubtedly bound to the iron. Some other ferric porphyrins prepared also contain one water ligand.



(65)

d) The reaction of the models with oxygen

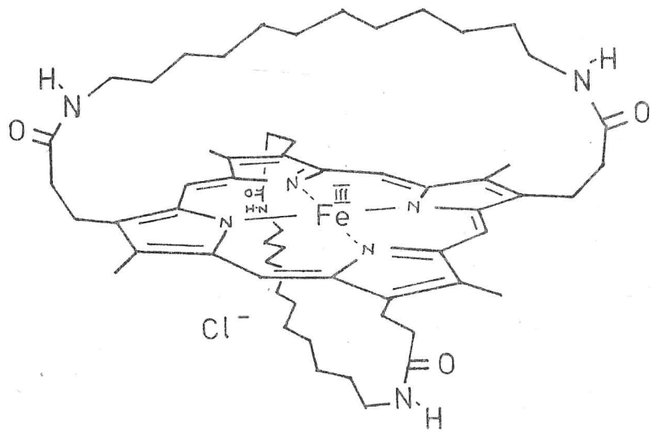
The test of the oxygen-binding capabilities of the two new models followed the procedures described in Chapter 2, section (d). The bridge plus tail model (65) gave visible spectra which differed in one important respect from those obtained previously. This molecule is unable to achieve the six-coordinate "haemochrome" configuration, because only one π-donor ligand is available per iron atom. The spectra that were obtained are shown in Figure 20.



Compound (65) in tetrahydrofuran at 20° (after reduction)
(a) ferrous
(b) carbon-monoxy haemochrome
(c) oxygen admitted

Figure 20

The compound was not very soluble in acetone, so tetrahydrofuran was used for these experiments. It was confirmed that this solvent gives no appreciable shifts in absorption maxima compared to acetone. The reduction was accomplished, as before, with aqueous sodium dithionite. In this case, to avoid problems of protonation of the tail, a phosphate buffer of pH 7.5 was used for the reductant. This produced the spectrum of Figure 20 (a), with

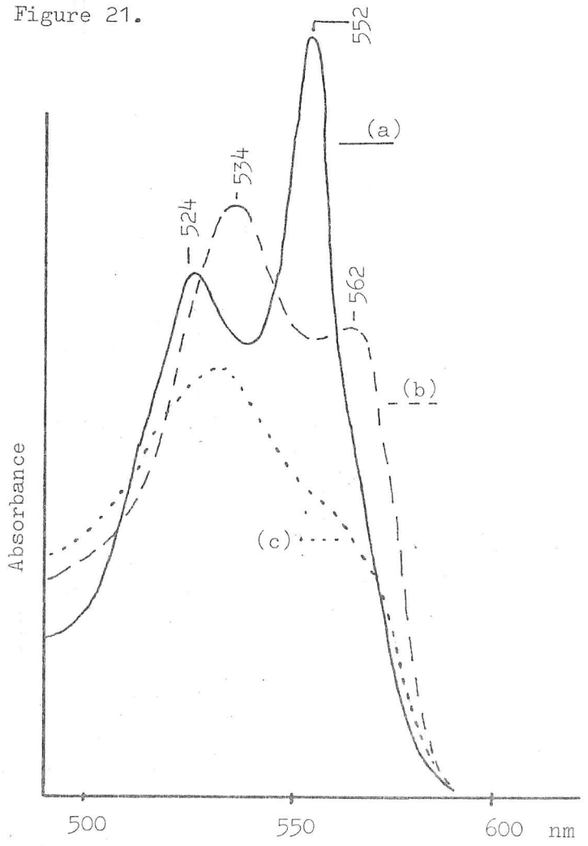


(74)

one broad peak having $\lambda_{max.}$ 548 nm, which corresponds to a high-spin five-coordinated species (compare Figure 10(a), p. 24). Myoglobin reconstituted with mesoporphyrin IX gives a peak at 546 nm under similar conditions [94]. Addition of carbon monoxide produced the spectrum of Figure 20 (b), with $\lambda_{max.}$ 532 and 560 nm; essentially identical to all the other carbon-monoxy haemochromes — six coordinate and low spin.

However, addition of oxygen to the five-coordinate ferrous form immediately converted the spectrum to one having $\lambda_{max.}$ near 520 nm and at 630 nm. These are the peaks of the now-familiar ferric form of the metalloporphyrins, and can equally be produced when (65) in tetrahydrofuran is examined without prior reduction.

It was discovered that the double bridged model (74) suffered the same fate when taken through the set of reactions whose spectra are shown in Figure 21.



Compound (74) in acetone at 20° (after reduction)

(a) haemochrome

(b) carbon-monoxy haemochrome

(c) oxygen admitted

Figure 21

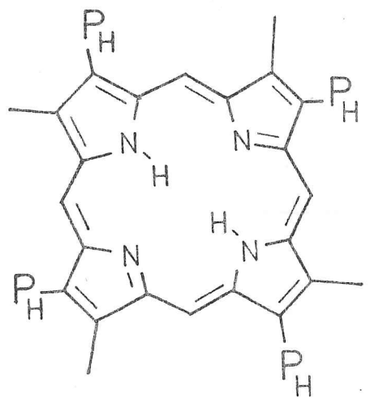
These spectra correspond exactly to those obtained with singly bridged models: the haemochrome can be produced and carbon monoxide attached, but oxygen produces instant oxidation.

The results presented here are for a typical series of reactions. Each was repeated to confirm its validity, and in addition other reagents and solvents were used for the reduction. In no case could any evidence be obtained for the existence of an oxygenated compound.

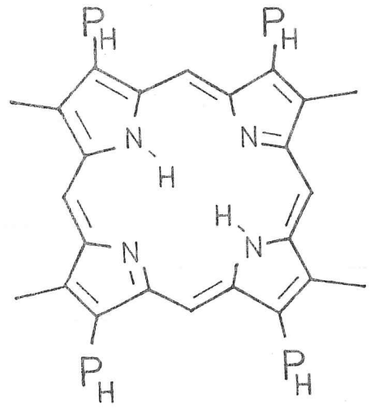
For example, an attractive method of producing an oxygen-bound species directly might be to add a superoxide (O_2^-) ligand to the ferric compounds. This would follow recent uses of superoxide ion as a combined reductant / oxygen source in porphyrin studies [95, 96], although it does not appear to have been used before for generating iron oxygen adducts in model studies.

Potassium superoxide is slightly soluble in dimethylsulphoxide or dimethylformamide in the presence of a crown ether — [18]-crown-6 [97] was used in the present studies. Unfortunately, no successful results were found. In either solvent, the various models gave spectra which were not consistent with adduct formation. The pattern of peaks showed that the compounds remain in the ferric form, and presumably the only reaction which occurs is to convert some models (used as their chloride salts) into their μ-oxo dimers.

An assessment of possible reasons for the failure to obtain oxygen adducts in any of the model studies is given in Chapter 6.



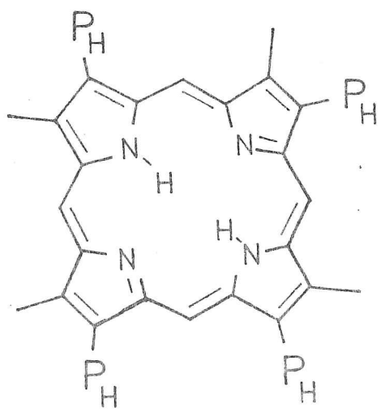
I



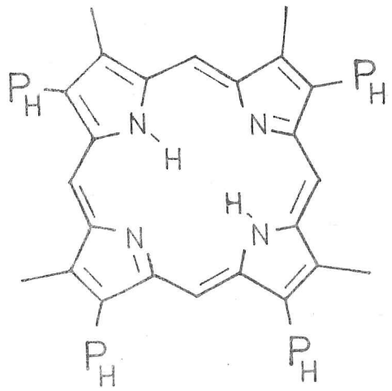
II

Coproporphyrins

III



IV



CHAPTER 4

Nuclear Magnetic Resonance studies

a) Introduction

The routine use of nuclear magnetic resonance (n.m.r.) spectroscopy has led to important structural insights in organic chemistry [98]. Few practising chemists will feel confident of the identity of newly-prepared compounds until they have studied the proton n.m.r. spectrum.

The first published spectrum in the porphyrin field was that of coproporphyrin I tetramethyl ester, in 1959 [99]. The earliest spectrum displayed the meso protons at about 10 δ and the central NH protons at -3.9δ, a wider range than that commonly observed in organic compounds (other than metal complexes). The power of the technique was immediately recognised. The large ring current in the macrocycle acts as an additional source of chemical shift, and for example, the resonance for the NH protons occurs at about 13 ppm to higher field than in pyrrole.

A recent comprehensive review of the n.m.r. of porphyrins by H. Scheer and J. J. Katz [100] covers the progress that had been made up to about 1974. The proton spectra are strongly solvent, concentration, and temperature dependent. In early work, the concentration dependence, due to aggregation under the influence of π - π interactions, was a source of confusion, for published work often neglected to report the concentration used. However, spectra taken with trifluoroacetic acid (t.f.a.) as solvent do not display aggregation effects (dication formation breaks down the aggregates); and studies on the concentration dependence of the chemical shifts in chloroform led to the useful observation that coproporphyrins III and IV could be distinguished by their behaviour [101].

As pulsed Fourier transform spectrometers have become commonly available, the limitation of having to operate at low concentrations to avoid dimer formation, in solvents other than t.f.a., has been largely removed [102].

The improved signal-to-noise ratio of these machines allows satisfactory proton spectra to be obtained in a reasonable time from solutions having concentrations in the 10^{-2} to 10^{-4} molar range. Furthermore, the low solubility of many porphyrins is no longer a problem when such sensitivity is available.

Fourier transform spectrometers have also been the key to ^{13}C n.m.r. experiments, which have found many applications in porphyrin studies, notably in biosynthesis, where the use of isotopically labelled materials has allowed selected carbon atoms to be followed as enzymatic transformations are carried out [6]. The ring current effects on the chemical shifts are less important in ^{13}C work, having the same absolute magnitude as in the proton case, which is now a smaller proportion of the total observed shift. Carbon n.m.r. at natural abundance still requires relatively large quantities of material (typically about 100 mg of a compound having a molecular weight of 500), and so the limiting factor is often its availability. No ^{13}C studies have been undertaken in the present work.

In choosing a set of conditions for obtaining proton spectra, a number of factors had to be considered. Firstly, a standard low concentration had to be chosen in order to avoid aggregation problems and to allow spectra to be recorded for the small quantities that might be available, particularly in a project where multiple products of a synthetic reaction might have to be analysed. The Liverpool group recommend [103] the use of porphyrin zinc chelates in solvents containing pyrrolidone to circumvent aggregation problems (especially in ^{13}C work), but this requires that derivatives of the porphyrins have to be made, which would be inconvenient.

Two spectrometers were available: a Varian CFT 20 (an 84 MHz instrument in proton work) and a 100.1 MHz Varian XL-100. It was readily apparent that a continuous wave machine did not have the sensitivity needed for the routine study of porphyrins in the range of concentrations accessible. The 84 MHz instrument was used for preliminary experiments, for example in identifying

components of a reaction mixture after chromatography, and the 100 MHz machine for building up a library of spectra of pure compounds. The concentration used for the latter studies was 0.013 M: that is, 4 mg of a compound of molecular weight 600 in $\frac{1}{2}$ ml solvent in a 5 mm diameter n.m.r. tube. This standard concentration was a balance between conflicting pressures: a need to have a large quantity to allow spectra to be quickly accumulated, and a desire to minimise the amount of synthetic material that was required, for the reasons mentioned above. With this concentration, spectra of reasonable clarity could be obtained after accumulation of as few as 50 transients (less than 4 minutes of machine time) and were essentially noise-free after 300 transients (20 minutes).

Secondly, a solvent had to be chosen which would dissolve the requisite quantity of porphyrin, even in the case of the free acids studied. These were quite insoluble in chloroform, which would otherwise have been the natural choice. Trifluoroacetic acid was an obvious alternative solvent — it has been widely used before for insoluble porphyrins — but has two important disadvantages. As a powerful acid, it could potentially react with some of the functionalities that were to be present in the range of compounds examined. It would also necessitate a neutralisation and extraction procedure to recover the compounds after the spectrum had been run, and this would be particularly inconvenient for the lactones and esters, which would probably be susceptible to hydrolysis under those conditions.

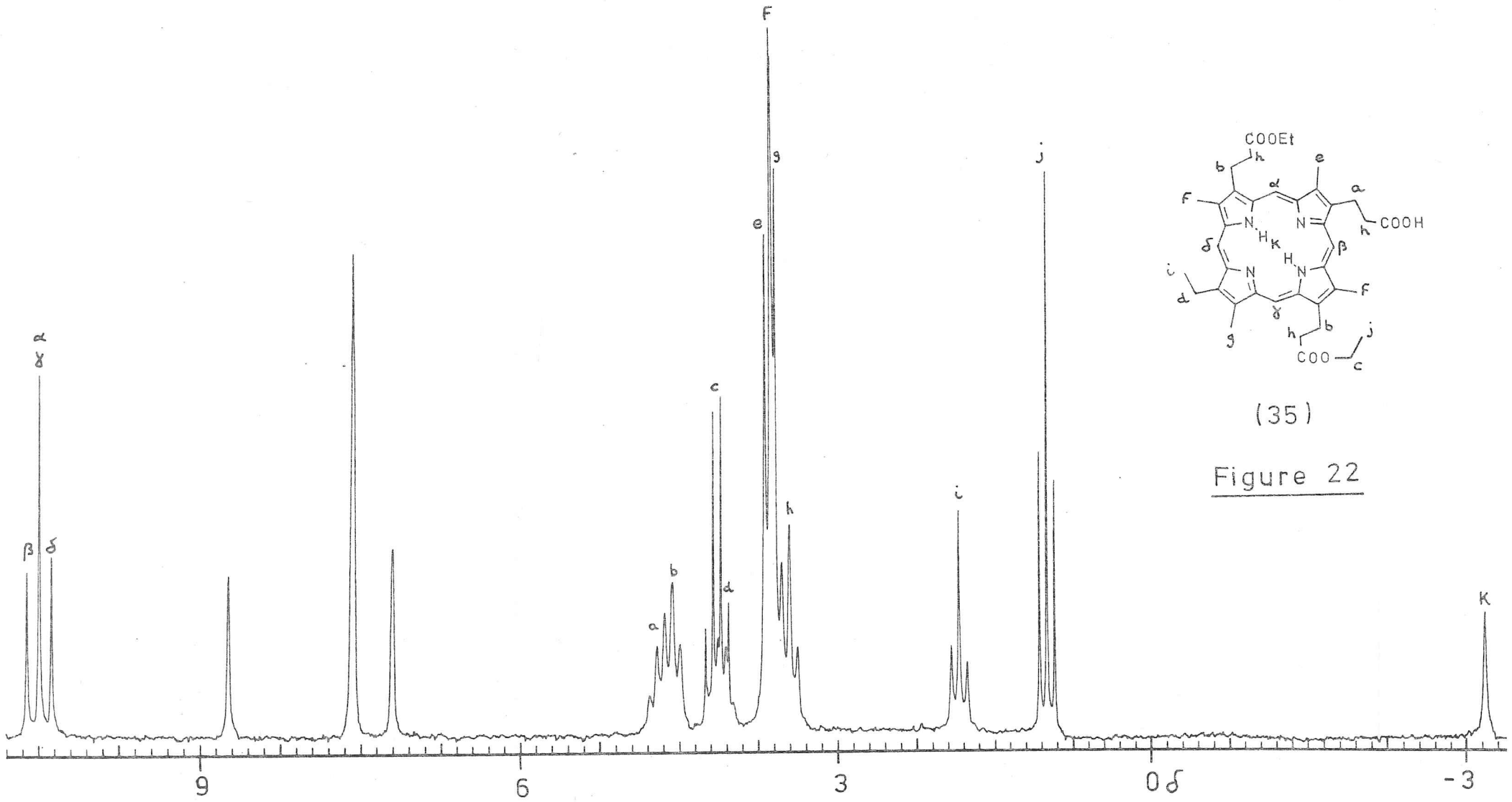
With these points in mind, it was decided to take routine spectra in deuteriopyridine, a solvent which has several advantages and only a few drawbacks. It is an excellent solvent for virtually all porphyrins, at least at concentrations less than 0.02 M. In addition, the signals from the residual protons in the deuterated material fall in the range 7 to 9 δ, which is free of porphyrin signals in most cases. Also, pyridine is readily removed by evaporation.

One disadvantage is that pyridine, unlike chloroform, is hygroscopic, and it is very difficult to avoid the presence of traces of water in the sample, which occasionally may mask some significant resonance. Since pyridine has chemically distinct sets of protons, the lock signal (a deuterium lock on the Fourier transform instruments) must be selected with care to ensure that the spectrometer is consistently locked on to the same signal so that all spectra are strictly comparable. Neither of these considerations is very important in practice, however, and pyridine has been found a most useful solvent.

There are no reports in the literature concerning the use of deuteriopyridine as solvent for free-base porphyrin n.m.r., although it has been used for metalloporphyrins, when its coordinating power is significant [104]. The absence of such work may be due to the very low solubility of protoporphyrin IX dimethyl ester in pyridine at 20°, although the spectrum may be run at 80° (vide infra). This observation was quite surprising in view of the compound's ready solubility in chloroform, and would discourage the general use of pyridine as solvent for biologically significant porphyrins.

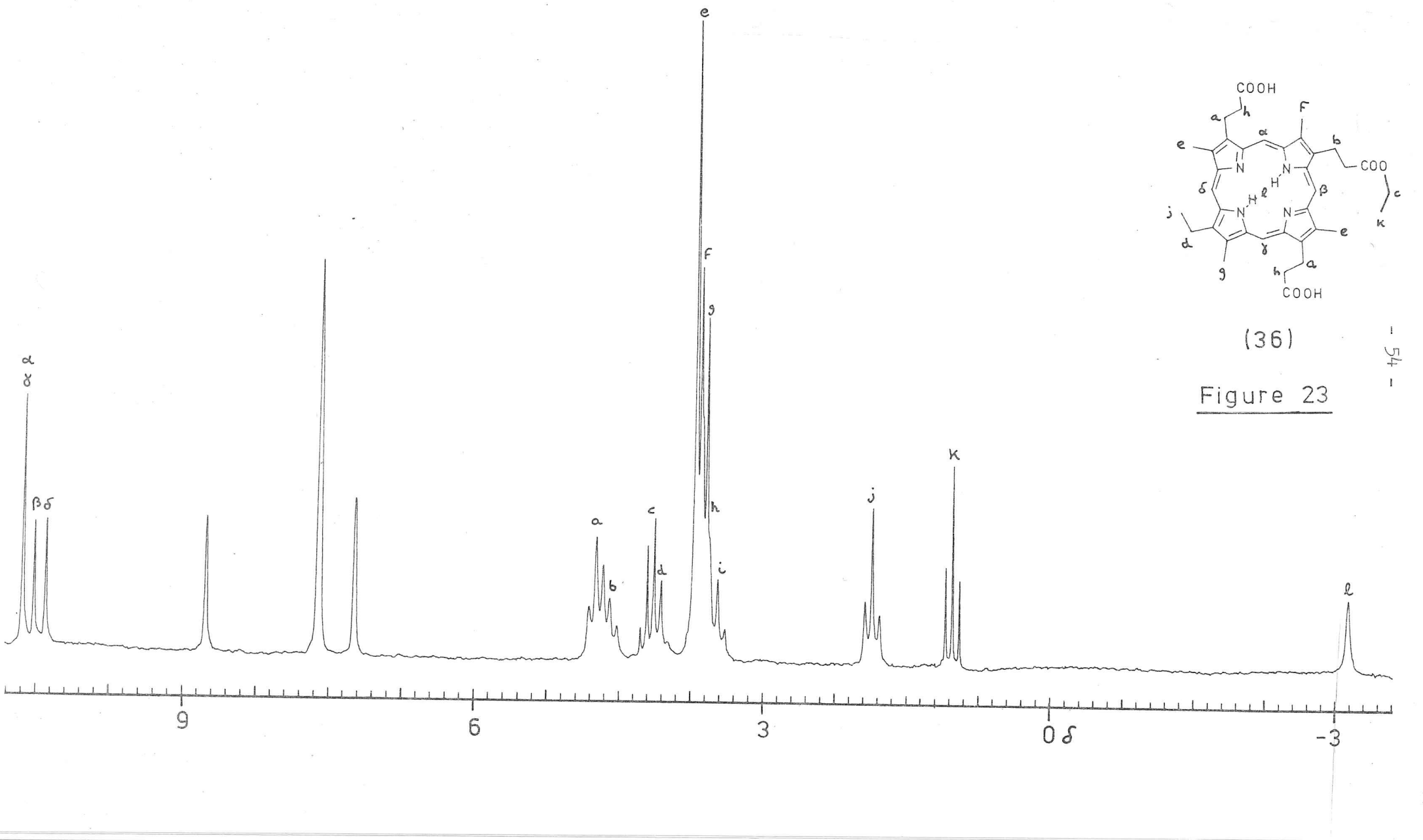
In addition, pyridine is not a suitable choice when lanthanide shift reagents are to be added to the sample to investigate their effect in shifting the resonances, for pyridine will strongly compete with the porphyrin for coordination to the metal.

Two main types of spectral result will now be presented. The first concerns the general features of all the porphyrins studied in pyridine, and the second will focus on spectra of bridged porphyrins, which are of special interest.



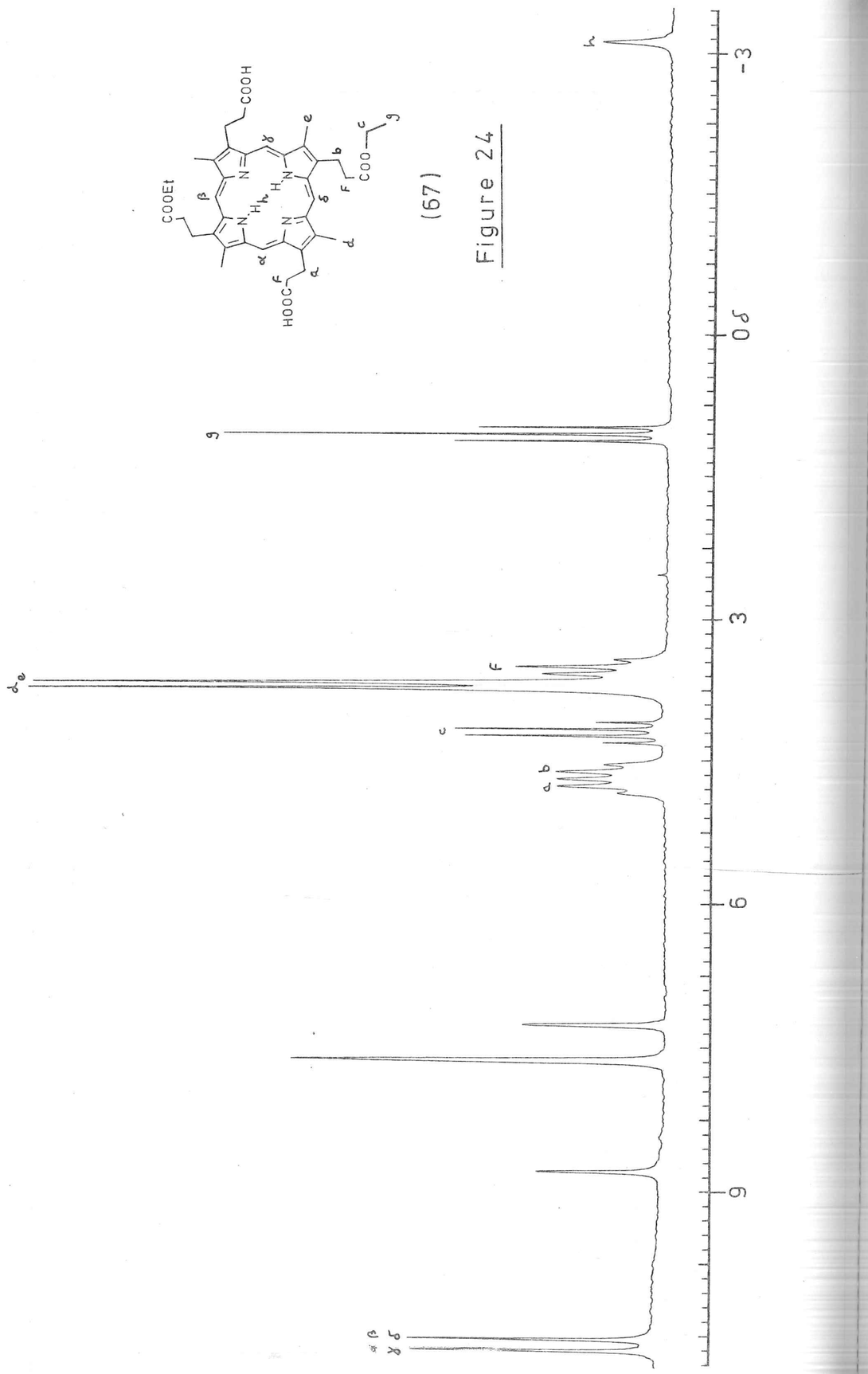
(35)

Figure 22



(36)

Figure 23



(67)

Figure 24

b) The proton n.m.r. of porphyrins in pyridine

The proton n.m.r. spectra of most of the porphyrins prepared in the current work have been examined in deuteriopyridine. The spectra obtained form a series from which virtually all the signals may be uniquely assigned, even the individual meso protons in unsymmetrical compounds. The assignments are a self-consistent set, as will now be detailed.

As a starting point, it is instructive to consider the spectra displayed in Figures 22 to 24. The full assignments are given in each Figure (and numerical values at the relevant points in the experimental section, Chapter 5). Many of the assignments are trivially made on the basis of multiplet structure and chemical shift [98, 100]. For example, the signal for the terminal methyl group of the ethyl esters is a triplet at 1.01 δ in all the compounds. However, subtle features require some justification. The individual singlets for the meso protons have been assigned according to the following scheme. Where a meso proton is flanked by two alkyl groups (e.g. the δ-protons in (35) and (36)), a signal is observed at 10.34 δ. When it is flanked by one alkyl group and one propionate ester, the resonance appears at 10.45 δ, and when, finally, it is flanked by an alkyl group and a propionic acid, the signal is at 10.56 δ.

Each of these (and other) chemical shift values are estimated to be accurate to $\pm$ 0.01 δ. It was shown on the XL-100 spectrometer that spectra were reproducible to that accuracy over the whole period of the experiments (1 year), provided that the concentration and temperature (28°) used were maintained constant. The machine is provided with a digital print-out to list relative chemical shift values, and these are produced as δ values given to three decimal places. Under the conditions of the experiments, with a spectral width of 1536 Hz and 8K data points in the computer memory at acquisition, one data point covers 0.37 Hz (1536/($\frac{1}{2}$ x 8192)) on printout: hence the error solely due to digitalisation may be up to $\pm$ 0.002 δ units.

With these values in mind, it is clear that the separation between the meso protons is of significance. One flanking propionate ester group moves the resonance 0.11 δ to lower field than that observed for a flanking alkyl group, and a propionic acid causes a 0.22 δ shift to lower field. Such additive effects on chemical shifts have been reported before for porphyrins in t.f.a. [56,100], and are in sharp contrast to the signals that are found in chloroform as solvent, when the meso protons often appear as a broad unresolved hump at about 10 δ. The authors of the earlier report noted that the additive effects were not maintained when a wider range of substituents (for example, ring formyl or ester groups) were studied [105]. However, in the present work, all the compounds have either alkyl or propionate attachments, and the shifts are consequently reproducible. The precise additive effects break down only in some of the bridged porphyrins.

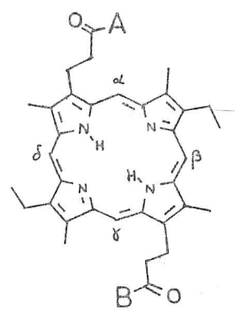
Table 2 lists the values of the chemical shifts for the meso protons in the compounds examined, and their assignment to the α, β, γ or δ positions. In some cases, marked by an asterisk, the resonances were resolved but their assignment is in doubt, usually because two very similar shifts were found. The numbers are presented to three decimal places, but, as detailed above, are only reliable within a single entry to $\pm 0.002\,\delta$ and between entries to $\pm 0.01\,\delta$.

In order to provide chemical shift values based on tetramethylsilane (T.M.S.), some spectra were run in deuteriopyridine containing T.M.S. The chemical shifts in others have been offset so the resonances for residual protiopyridine appear at 8.700, 7.55, and 7.18 δ, the first value being taken as standard in those cases where any discrepancy exists between the three pyridine signals. These values for the pyridine agree with those found when T.M.S. was used, and again, were found to be reliable to within $\pm 0.01\,\delta$.

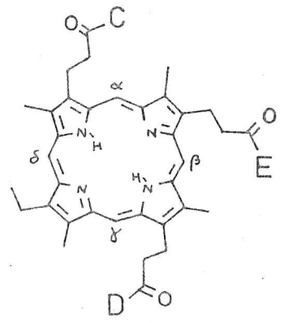
Further systematic differences are apparent in the spectra. The methylene adjacent to the porphyrin in a propionate chain gives a triplet at

Cpd.	A and B
(16)	OMe
(19)	OH
(21)	$O(CH_2)_4C{\equiv}CH$
(21a)	OC_6H_{13}
(29)	$N(Me)(CH_2)_5COOMe$

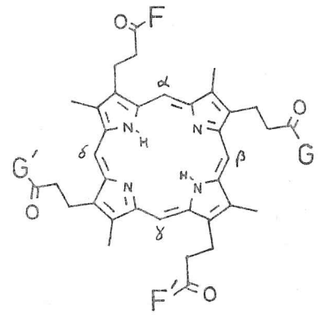
Cpd.	A to B
(23)	$-O(CH_2)_{12}O-$
(31)	$-NH(CH_2)_{12}NH-$
(32)	$-O(CH_2)_{10}O-$
(33)	$-O(CH_2)_9O-$
(34)	$-O(CH_2)_8O-$



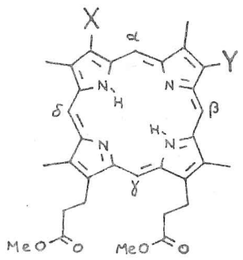
Cpd.	C	D	E
(35)	OEt	OEt	OH
(36)	OH	OH	OEt
(36a)	OEt	OEt	OEt
(55)	OEt	OEt	$NH(CH_2)_3$-Imidazole
(56)	OH	OH	$NH(CH_2)_3$-Imidazole
(57)	$-NH(CH_2)_{12}NH-$		$NH(CH_2)_3$-Imidazole
(63)	$-NH(CH_2)_{12}NH-$		OEt
(64)	$-NH(CH_2)_{12}NH-$		OH



Cpd.	F	F'	G	G'
(67)	OH	OH	OEt	OEt
(68)	OH	OEt	OEt	OEt
(69)	OEt	OEt	OEt	OEt
(71)	$-NH(CH_2)_{12}NH-$		OEt	OEt
(72)	$-NH(CH_2)_{12}NH-$		OH	OH
(73)	$-NH(CH_2)_{12}NH-$		$-NH(CH_2)_{12}NH-$	



Cpd.	X	Y
(XI)	$CH{:}CH_2$	$CH{:}CH_2$
(X)	CH_2CH_2COOMe	CH_2CH_2COOMe



Key to structures of compounds for Table 2.

Table 2 Meso proton chemical shifts (all are $(10+\varkappa)\delta$, with $\varkappa$ listed).

Compound No.	α	β	γ	δ
(16)	.445	.347	.445	.347
(19)	.576	.344	.576	.344
(21)	.454	.346	.454	.346
(21a)	.463	.344	.463	.344
(23)	.464	.304	.464	.304
(29)	.579	.382	.579	.382
(31)	.605	.315	.605	.315
(32)	.479	.310	.479	.310
(33)	.411	.320	.411	.320
(34)	.400	.337	.400	.337
(35)	.448	.562	.448	.336
(36)	.565	.452	.565	.335
(36a)	.446	.446	.446	.328
(55)	.461*	.526	.417*	.341
(56)	.551*	.573*	.532*	.335
(57)	.561*	.663	.538*	.310
(63)	.633*	.421	.591*	.311
(64)	.617*	.513*	.558*	.303
(67)	.556	.440	.556	.440
(68)	.543	.432	.432	.432
(69)	.430	.430	.430	.430
(71)	.616	.409	.616	.409
(72)	.574	.492	.574	.492
(73)	.591	.591	.591	.591
(X)	.428*	.443*	.562	.306
(XI)	.387*	.427*	.427*	.263

4.69 δ when the propionic acid is present, and at 4.55 δ when it is in the form of its ester. Similarily, the methyl group on the same pyrrole unit as a propionic acid gives a signal at 3.67 δ, while that adjacent to an ester has a chemical shift of 3.62 δ. In contrast, the methyl group next to a simple ethyl substituent resonates at higher field, typically 3.56 δ.

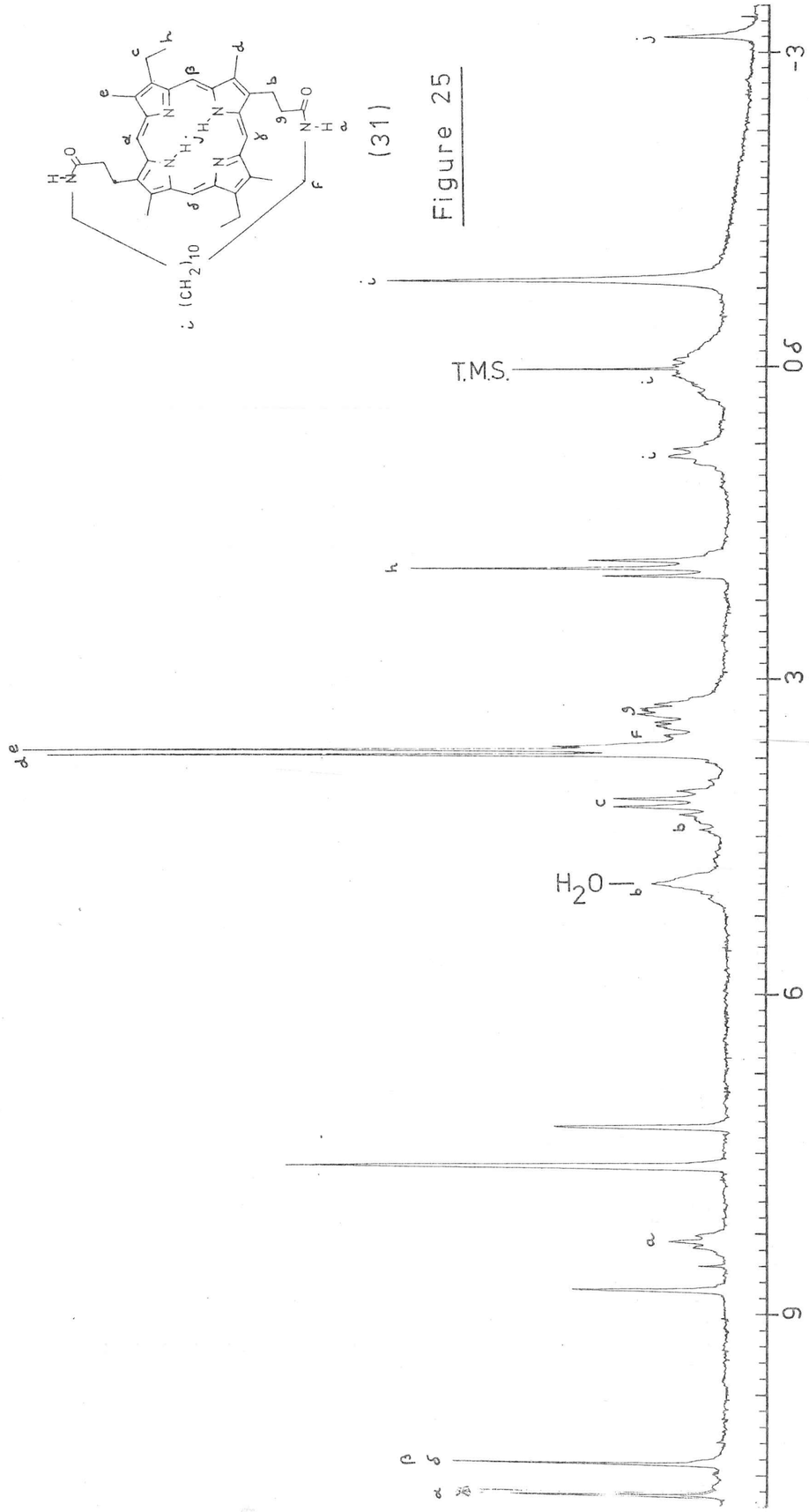
In all examples, the general trend is for chemical shifts to vary in the order acid, ester, alkyl, from low to high field; and this has provided a useful identification in those cases where a reaction produced a mixture of products, for the desired compounds could often be picked out, particularly on the basis of the meso proton resonances.

A few porphyrins of biochemical significance were also examined, and these results are included in Table 2. The most interesting finding is that in coproporphyrin III tetramethyl ester (X), the resonances for the γ and δ meso protons are clearly distinguished from those of the α and β positions. In accord with the additive nature of the chemical shifts, the γ proton, flanked by two esters, gives a signal at 10.56 δ, while the δ proton, flanked by two alkyl groups, comes at 10.31 δ. The other two protons lie at 10.42 and 10.44 δ.

The effect of changes in concentration on the spectra in pyridine was also briefly explored. Mesoporphyrin II dimethyl ester (16) was taken at three different concentrations: $\frac{1}{2}$ mg, 4 mg, and 30 mg samples were used, the latter at 80° to ensure its complete solubility in the $\frac{1}{2}$ ml of solvent. The results for the meso protons (which were most subject to change) are shown in Table 3. The δ values are based on pyridine-d_4 at 8.700 δ.

Molar concentration (mM/l)	α, γ	β, δ
1.7	10.469	10.368
13.5	10.446	10.346
100 (80°)	10.339	10.255

Table 3



It is seen that the shifts, while significant, are not larger than 0.15 δ in the range of concentration (and temperature) used. The spectra were all qualitatively similar, resonances moving to higher field as the concentration increased. This work emphasises the need for a standard concentration in porphyrin n.m.r. studies, but confirms that in pyridine at concentrations likely to be accessible within solubility and spectrometer limits, no unpredictable aggregation effect will result.

c) The proton n.m.r. of bridged porphyrins

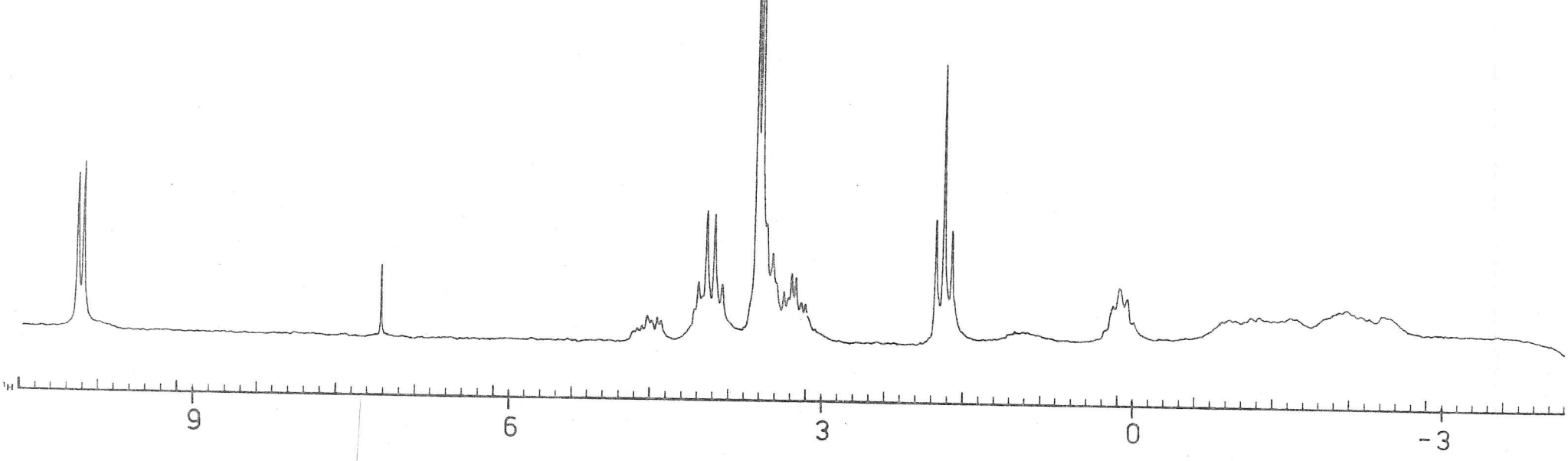
The proton n.m.r. of the dodeca-1,12-diamine bridged porphyrin (31), with T.M.S. as an internal reference, is reproduced in Figure 25. In addition to signals from 1.8 to 11 δ assigned to various components of the porphyrin and its substituents (as shown in the Figure), a series of signals appears at higher field. These are assigned to the protons of the bridge: the observed upfield shifts are due to the ring current in the macrocycle which it spans. The effect of the porphyrin ring current on protons above the plane of the aromatic molecule has previously been observed (mainly in metalloporphyrins), and is reviewed by H. Schleer and J. J. Katz [100]. These authors also review the theoretical models that have been proposed to quantify the effect to be expected on the chemical shift of a proton at a given point above the porphyrin.

The n.m.r. spectra of the diol bridged porphyrins (32), (33), and (34) in $CDCl_3$ are shown in Figures 26 to 28. It has been noted that some spectra are strongly solvent-dependent, and each of the bridged porphyrins has been examined in both $CDCl_3$ and deuteriopyridine. Table 4 lists the observed chemical shifts in the bridges for representative cases. As can be seen from Figures 25 to 28, the resonances for a given methylene group in the chain often appears as a broad unresolved signal, reflecting the many couplings to other protons present.

C_{10} Bridge

(32)

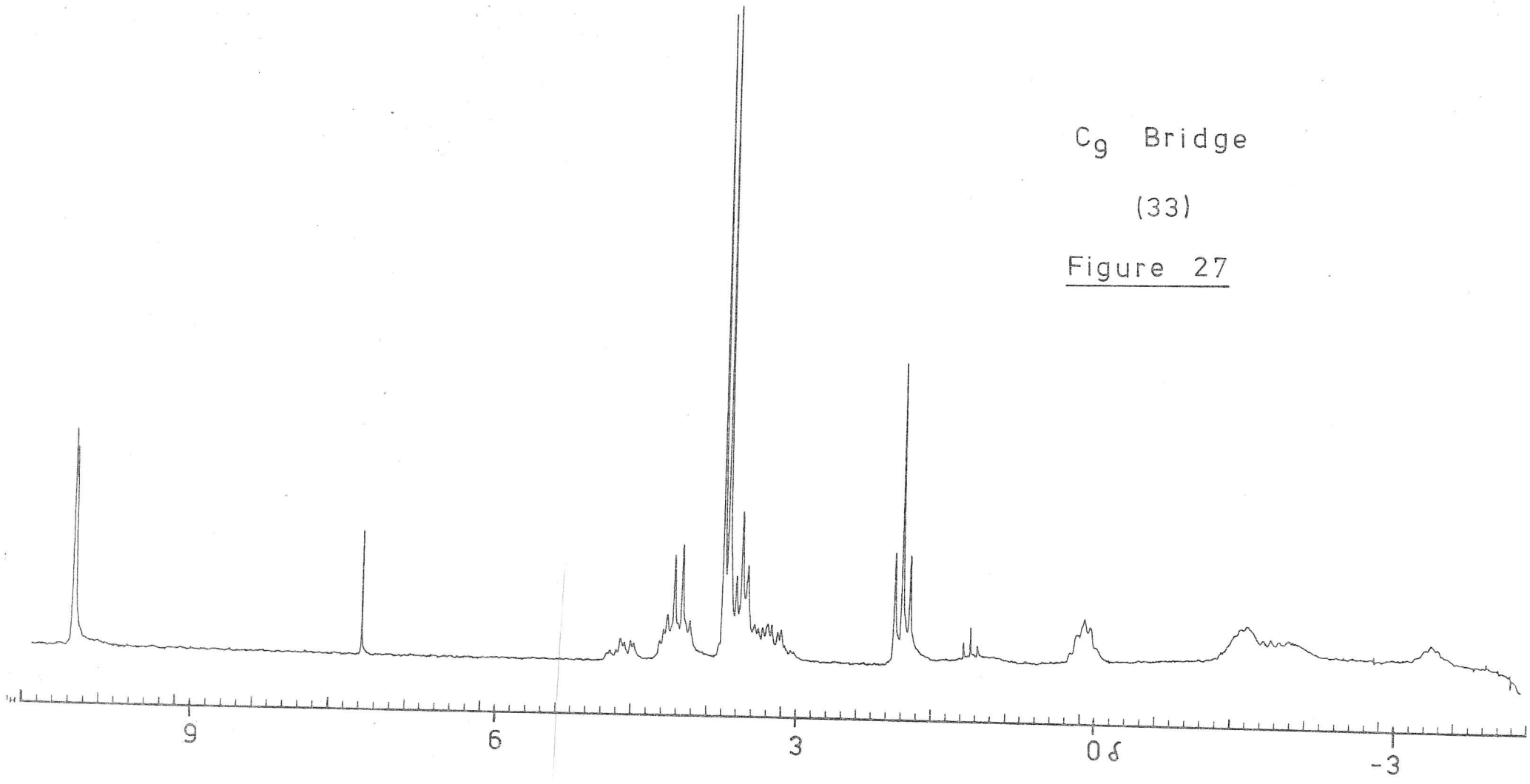
Figure 26



C_9 Bridge

(33)

Figure 27



C_8 Bridge

(34)

Figure 28

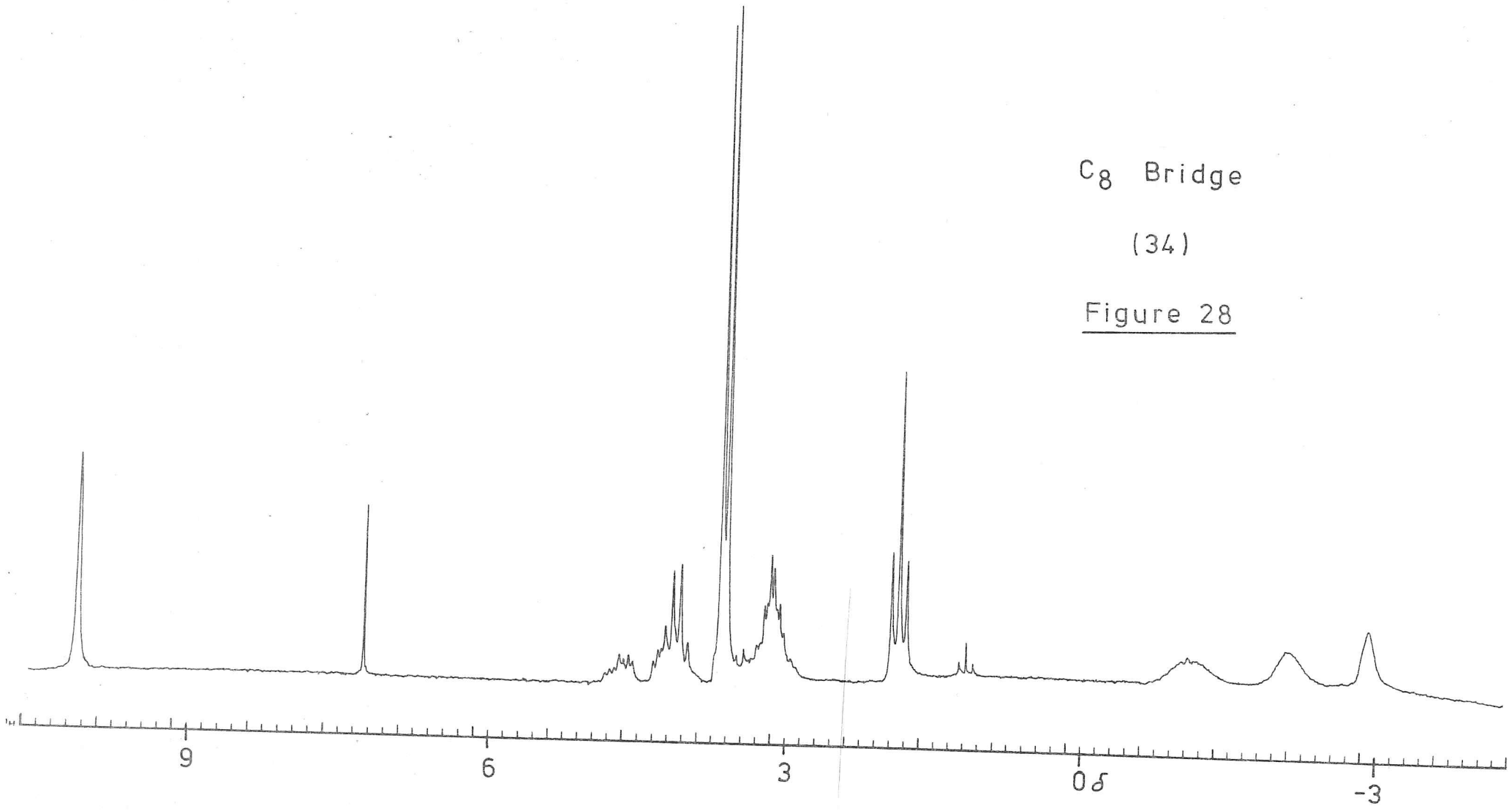


Table 4 Chemical shifts of bridge protons (in pyridine and chloroform) (in δ).

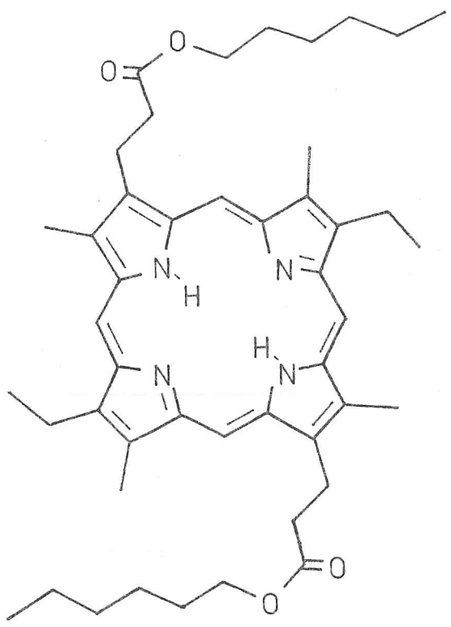
Nomenclature: Porphyrin $-\overset{O}{\overset{\|}{C}}-X-\overset{1}{CH_2}-\overset{2}{CH_2}-\overset{3}{CH_2}-\overset{4}{CH_2}-\overset{5}{CH_2}-\overset{6}{CH_2}--$

Compound	C_1		C_2		C_3		C_4		C_5		C_6	
	Pyr.	$CDCl_3$	Pyr.	$CDCl_3$	Pyr.	$CDCl_3$	Pyr.	$CDCl_3$	Pyr.	$CDCl_3$	Pyr.	$CDCl_3$
C_{12}-lactam (31)	3.56	2.62	0.78	0.38	0.2 to -0.2	-0.04	-0.85	-0.45	-0.85	-0.45	-0.85	-0.45
C_{12}-lactone (23)	3.90	3.69	0.82	0.75	0.2 to -0.2	-0.10	-0.95	-0.71	-0.95	-0.71	-0.95	-0.71
C_{10}-lactone (32)	3.86	3.58	0.30	0.21	-0.72	-1.0	-1.4	-1.8	-2.1	-2.3		
C_9-lactone (33)	3.63	3.49	0.15	0.08	-1.76	-1.88	-1.46	-1.52	-3.18	-3.4		
C_8-lactone (34)	3.25	3.18	-0.9	-1.0	-1.9	-2.0	-2.76	-2.83				
Double Bridge (73)	3.40	2.77	0.80	0.47	0.2 to -0.2	0.06	0.2 to -0.2	0.06	-0.60	-0.20	-0.60	-0.20

J. C. Waterton has examined the magnesium adducts of some of these porphyrins at 270 MHz, and has been able to measure many of the coupling constants under those conditions [106]. He has also performed decoupling experiments to assign definitively the resonances in the spectrum of the nine-carbon diol bridged porphyrin (33). As summarised in Table 4, the finding is that the high field shift shown by the signals from the bridge protons is not a simple function of their proximity to the axis of the porphyrin. Instead, the resonances for the protons attached to C_3 of the chain appear at higher field than those attached to C_4. One interpretation of this result is that the protons are influenced by the ring currents within individual pyrrole fragments and that a simple "single loop" model [100] for the ring current is therefore insufficient to explain the detailed pattern of chemical shifts. An alternative explanation might be that certain methylene groups are held in a conformation which places them closer to the plane of the ring than their neighbours [106].

Other assignments given in Table 4 have been made simply on the basis of the chemical shift data, so it is possible that subtle effects may also operate in cases other than the nine-carbon bridged compound to upset the quoted designations. The spectrum of the ten-carbon bridged porphyrin (32) is especially difficult to assign in the absence of decoupling results at 270 MHz.

As mentioned above, some bridged porphyrins show markedly different chemical shifts in the two solvents used. For example, the protons at highest field in (31) appear at -0.85 δ in deuteriopyridine, but only at -0.45 δ in $CDCl_3$. In order to have a "normal" value on which to base interpretation of these differences, the bis hexyl ester of mesoporphyrin II (21a) was prepared (see Chapter 5). The central alkyl protons in this compound resonate at 1.09 δ in $CDCl_3$ and at 0.85 δ in pyridine. The change of 0.24 δ in moving from pyridine to chloroform in the "normal" case has expanded to



(21a)

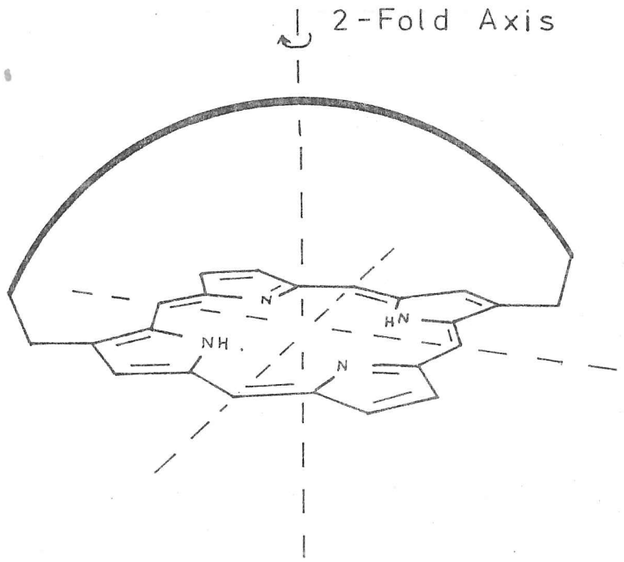


Figure 29

0.4δ in the C_{12} bridged (31). In contrast, the maximum difference in chemical shifts of the protons of the C_8 bridged (34) has shrunk to 0.1δ.

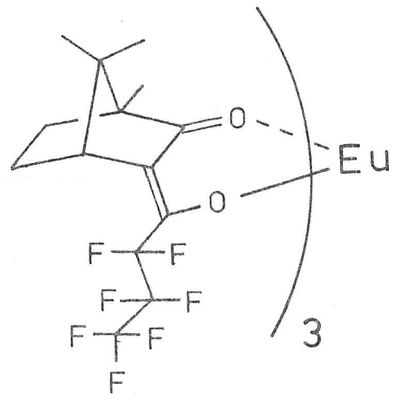
A possible explanation of this effect is that the configuration of a bridge may differ in the two solvents. By inspection of the molecular models of the respective porphyrins, it is seen that the twelve-carbon bridge is relatively free to take up a range of conformations over the macrocycle. It appears able to swing over a large portion of the plane of the porphyrin, and hence individual protons of the chain will sample a large section of the magnetic anisotropy caused by the ring current. Chloroform and pyridine will be expected to interact differently with the porphyrin (as evidenced by the meso protons, which show qualitatively different behaviour in each solvent), so the bridge may be in an alternative set of positions. The shorter bridge will be less able to vary its position relative to the porphyrin, for the molecular models reveal that it is quite tightly constrained near the macrocycle. Hence solvent effects will be less important in determining the observed chemical shifts.

All the singly-bridged porphyrins are chiral. Most have a two-fold rotation axis through the centre of the porphyrin at right-angles to its plane, but lack the mirror plane of unbridged molecules (see Figure 29). In theory, the bridged porphyrins could be resolved into enantiomeric forms, but this has not been attempted! However, it was of interest to demonstrate the chirality, for its observation is powerful evidence for the correctness of the structural assignments. N.m.r. provides a means for the examination of a racemic mixture [107]. The use of one enantiomer of a chiral solvent, or of a chiral shift reagent, opens up the possibility of observing a different signal from the two related diasteriomeric complexes that will result when these materials interact with the racemate.

The n.m.r. of the twelve-carbon diol bridged porphyrin (23) was recorded in $CDCl_3$ containing increasing amounts of the chiral lanthanide shift reagent

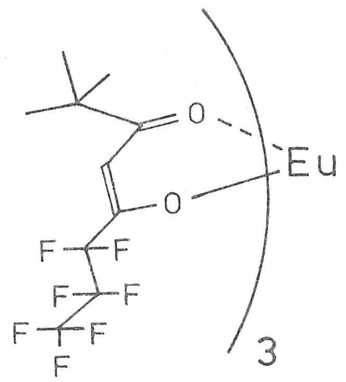
$Eu(hfc)_3$

(XII)



$Eu(fod)_3$

(XIII)

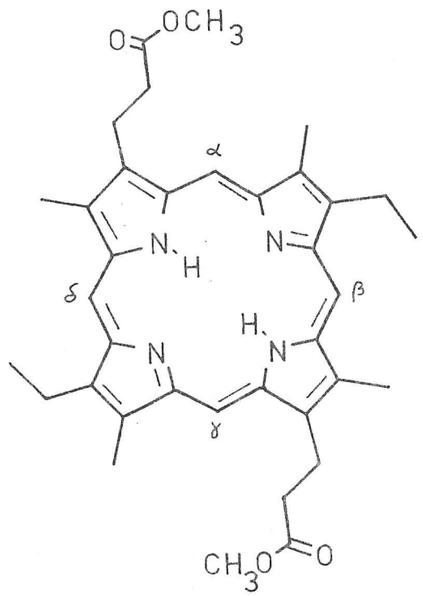


Tris-[3-(heptafluoropropylhydroxymethylene)-d-camphorato]-Europium, $Eu(hfc)_3$ (XII) [108] or its Praesodymium analogue $Pr(hfc)_3$ [109]. As a control experiment, the effect of the reagent (XII) on mesoporphyrin II dimethyl ester (16) was also examined. In addition, comparison was made of the achiral shift reagent $Eu(fod)_3$ (XIII) [110] acting on the porphyrins. The results are presented in Table 5, which gives the observed chemical shifts of the meso protons in each case. The meso proton signals are conveniently identified, as they appear well separated from other resonances.

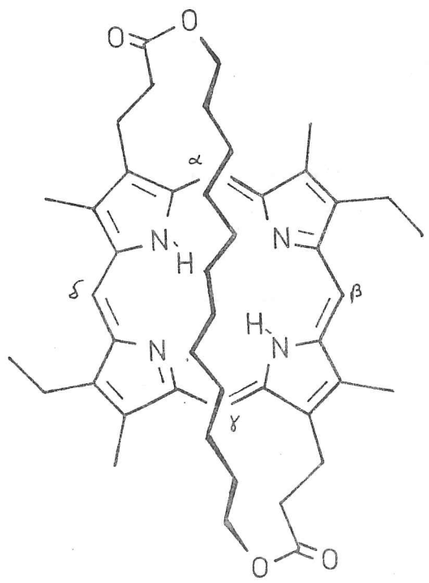
Compound (in $CDCl_3$)	Reagent (Amount)	Signals (δ)			δal Ratio
Mesoporphyrin II dimethyl ester (16) (4 mg)	None	10.00			-
	$Eu(fod)_3$ (5 mg)	10.18	10.71		1 : 1
	$Eu(hfc)_3$ (4.5 mg)	10.11	10.67		1 : 1
C_{12}-diol bridged porphyrin (23) (5 mg)	None	9.97	10.00		1 : 1
	$Eu(fod)_3$ (5 mg)	10.38	11.23		1 : 1
	$Eu(hfc)_3$ (4.5 mg)	10.41	11.71	11.95	2 : 1 : 1
	$Pr(hfc)_3$ (1 mg)	9.95	9.77	9.73	2 : 1 : 1
	$Pr(hfc)_3$ (3 mg)	9.80	9.23	9.13	2 : 1 : 1

Table 5 (Chemical shifts relative to $CHCl_3$ at 7.25δ)

The results can be rationalised by considering the likely effects of coordination of the ester carbonyl groups to the shift reagent. The signal from the meso protons at the α and γ positions, which are closer to the coordination sites, will experience the greater shift [110]. Thus, either $Eu(fod)_3$ or $Eu(hfc)_3$ can split the two types of signal in mesoporphyrin II dimethyl ester (16), which are moved downfield by different amounts. In



(16)



(23)

chloroform, these resonances are not resolved in the absence of shift reagent, although they are resolved in pyridine as solvent (see Table 2).

$Eu(fod)_3$ has a rather similar effect on the diol bridged compound (23). Here, the two pairs of meso protons are already (just) resolved in chloroform. The resolution is increased when shift reagent is added, but no further splitting is observed (nor is any expected with this achiral reagent). However, when $Eu(hfc)_3$ is employed, the signals from the pair of meso protons which are moved downfield to the greatest extent are also clearly separated into lines of equal intensity. Each signal arises from the α and γ meso protons in a particular enantiomer of the porphyrin and has half the intensity of the signals at higher field corresponding to the β and δ meso protons, which are still not resolved.

As a further confirmation of these results, a parallel experiment was carried out using $Pr(hfc)_3$. Praesodymium is known[110] to shift the signals of neighbouring protons in the opposite direction to that observed for Europium, so it was expected to shift the signals upfield. This was found to be the case, as shown in Table 5, and increasing the amount of the reagent increased both the shift and the resolution achieved between the diasteriomers. The results with the two chiral reagents are complementary, and each differs from the effect of the achiral lanthanide.

The conclusion which may be drawn is that a racemic porphyrin is present, and this evidence alone is firm support for the structure of the bridged porphyrin (23).

The non-equivalence of the methylene protons in the propionate side-chain of the bridged porphyrins (vide infra), confirms this picture, and shows that flipping of the bridge from one face of the macrocycle to the other (to put the enantiomers into equilibrium) is not a fast process.

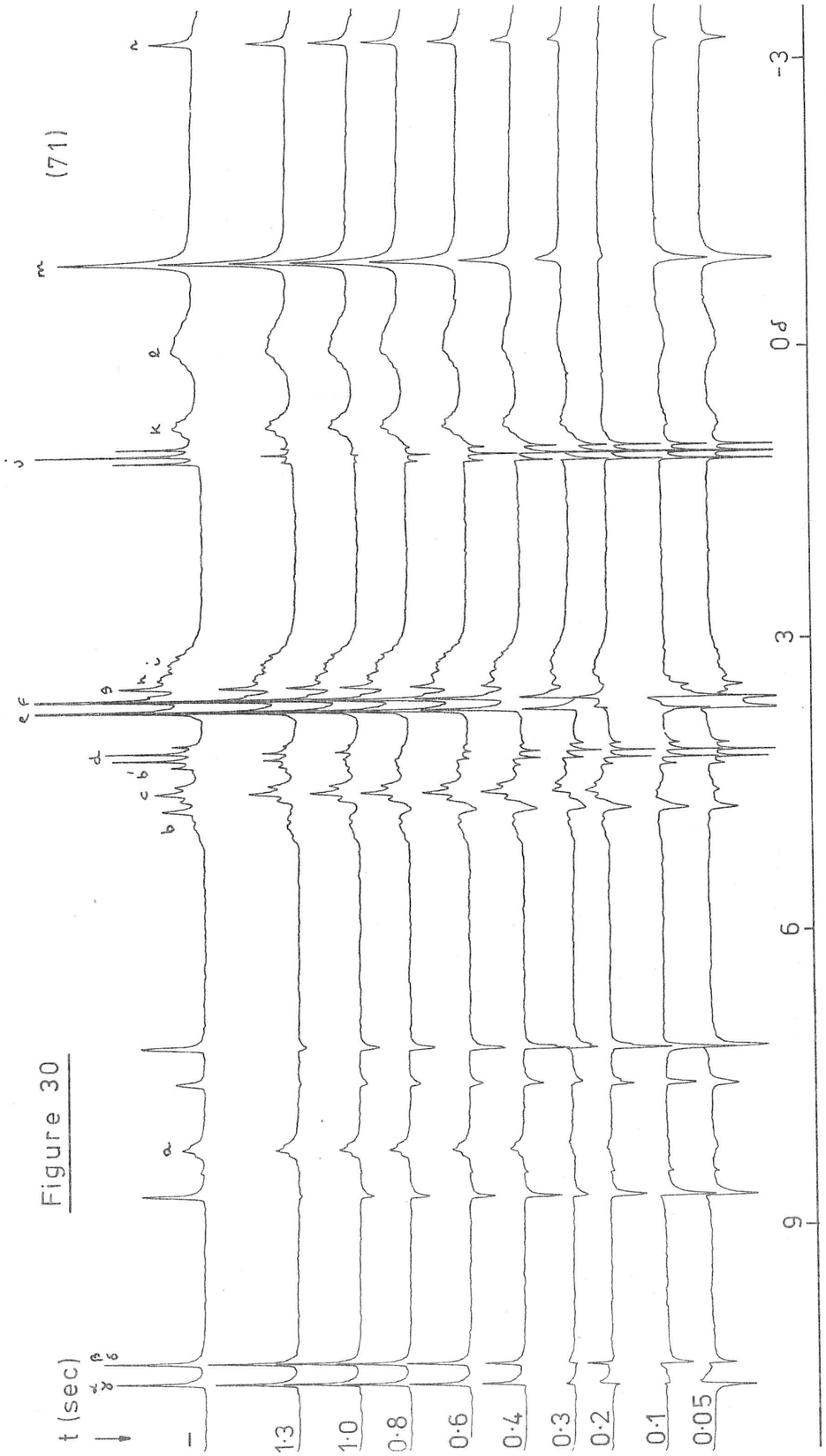


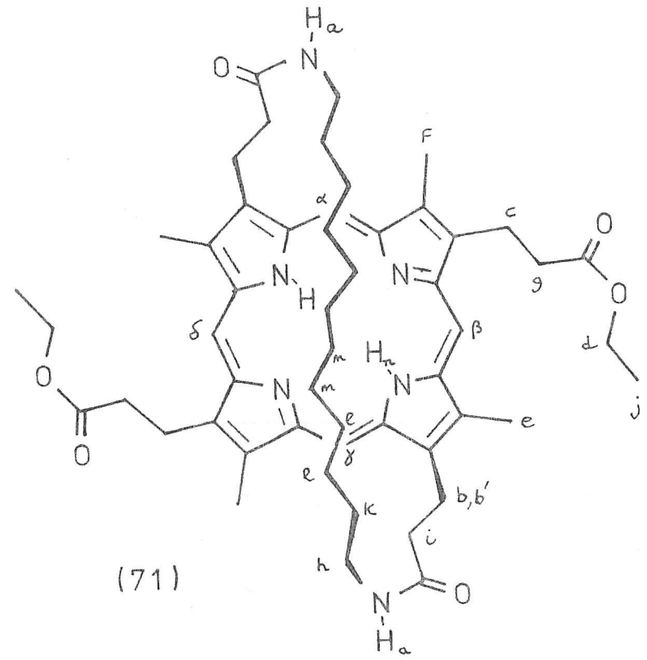
Figure 30

Two independent pieces of n.m.r. evidence have been presented for the bridged structures: the chemical shifts and the chirality. A third was the subject of considerable experimentation, namely the relaxation effects in the bridges, when compared to those in open structures.

The application of relaxation methods to structural studies has recently been reviewed by L. D. Hall [111], and some work in the porphyrin field has been described by J. K. M. Sanders and his group [112]. The use of pulsed Fourier transform spectrometers allows the routine determination of the spin-lattice relaxation time T_1 to be made in a short series of experiments. The simplest method requires a two-pulse sequence (180°-t-90°) where the pulses are separated by a time t which is varied in separate experiments — the "inversion-recovery" method [111]. A practical example will be taken to illustrate the important information which can be obtained from such a sequence: this is shown in the series of spectra of Figure 30. The "normal" spectrum of the bridged porphyrin (71) is displayed above others in which a 180° pulse has first been applied and the spectrum recorded (by action of a 90° pulse and acquisition of the free induction decay in the usual way) after a delay time t which is given alongside each spectrum in the Figure. Chemical shift assignments are shown in Figure 31.

Qualitatively, it is seen that for a short value of t, individual signals are inverted, and that as t is increased these signals decay, to be replaced by positive ones. At some time t_{null} a given signal will disappear from the recorded spectrum entirely, as the magnetisation due to that signal has relaxed back to zero at that time, and is therefore not sensed when the sampling 90° pulse is applied. For example, the signal at -3.1 δ (the NH protons) is inverted for $t < t_{null} = 0.2$ seconds and positive thereafter. This provides a simple measurement of T_1, for this is related to t_{null} by the expression [102]

$$T_1 = t_{null} \div \ln 2 .$$



(71)

Signal	δ	t_{null} (sec)
α, γ	10.62	0.2
β, δ	10.41	0.2
a	8.24	0.1
b, b'	4.1, 4.8	0.05
c	4.57	0.1
d	4.18	0.8
e	3.73	0.2
f	3.61	0.2
g	3.49	0.2
h	3.4	0.2
i	3.3	0.1
j	1.10	1.0
k	0.82	0.2
l	0.03	0.2
m	-0.87	0.2
n	-3.15	0.2

Assignments for spectrum of Figure 30, and measured null times.

Figure 31

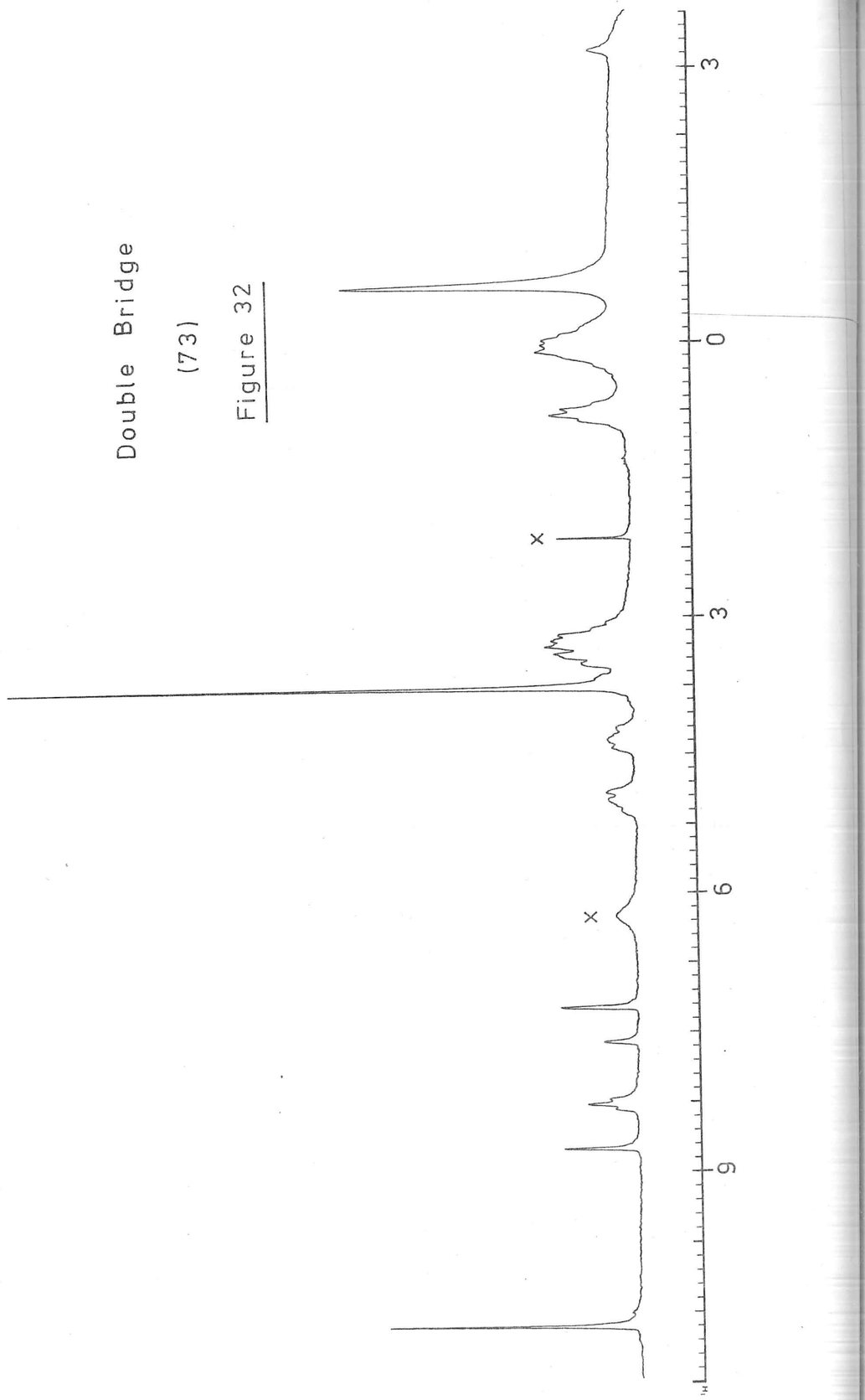
More accurate determinations of T_1 are possible if a graphical plot of the logarithm of the peak intensity versus t is made [102,111], but for the present largely qualitative work the null method is of sufficient precision. In addition, since porphyrins are large rigid entities, their protons have long correlation times τ and hence short T_1, for these terms for a given proton i are related by

$$1 / T_{1i} \propto \tau_i \cdot \sum_{i \neq j}^{j} r_{ij}^{-6}$$

where r_{ij} is the distance between the proton i and its neighbour j [102].

The shortness of porphyrin T_1 values means that measurements are less susceptible to errors caused by paramagnetic impurities (e.g. dissolved oxygen) and by the failure of the perturbed magnetisation to return to its equilibrium value between successive 180° pulses. The determinations were carried out with an acquisition time of 2.66 sec, but no pulse delay after acquisition before the next 180° pulse. In any case, relative null times within a single set of spectra such as that of Figure 30 have been found to be reproducible, and several interesting observations have been made.

First, it is seen that the signals from the protons of the bridge null at 0.2 sec, a time comparable to the null time of the NH and meso protons. In contrast, the signals due to the methyl and methylene protons of the ethyl ester null only after a much longer time (1.0 and 0.8 sec respectively). This finding is further evidence for the rigidity of the bridge, and hence for the molecular structure. The rotational freedom of an alkyl ester gives its protons a short correlation time τ, and hence a long null time when compared to the protons in the methylene chain of the bridge. The $\sum r_{ij}^{-6}$ term is similar for the two cases as this is dominated by the nearest-neighbour interactions.



Double Bridge

(73)

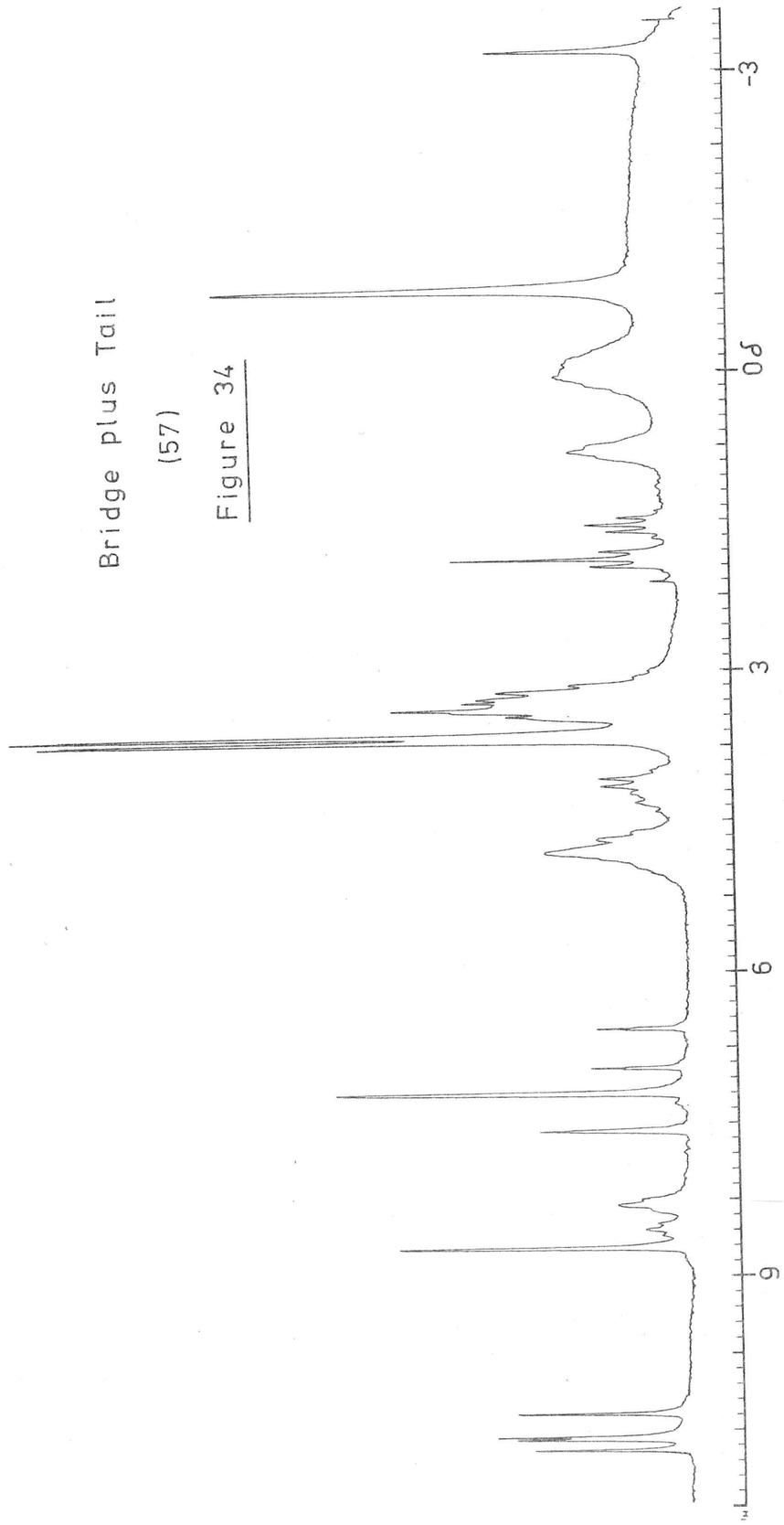
Figure 32

The bridge is tumbling at a rate comparable with that of the porphyrin framework, while the ester groups have additional rotational freedom. That is, $\tau_{bridge} \approx \tau_{macrocycle}$. It is apparent that a meso proton has fewer near neighbours than a proton on the bridge, so the Σr_{ij}^{-6} term for it is smaller. If the null times are to be approximately equal, this implies that the protons on the bridge have no more rotational freedom than the meso protons, which, of course, must tumble with the porphyrin framework.

A second useful application of relaxation methods is apparent in the spectra of Figure 30. Solvent signals can be nulled out of the spectrum, while the porphyrin signals have largely recovered their "normal" appearance. The small solvent molecules have relatively large T_1 values, and those in pyridine null at about 1.3 sec. More importantly, signals caused by the trace of water in the sample, which obscure the signal at 4.8 δ can be nulled (at 1.0 sec) to fully reveal the hidden resonances of the propionate side-chain, whose relaxation times are much shorter. This is a very useful additional aid, for even impure samples can be examined in this way to assist assignment of overlapping signals.

Lastly, the nulling of one part of the spectrum can reveal unexpected overlaps. Thus, at 0.8 sec in Figure 30 the methylene of the ethyl ester has been nulled (at 4.2 δ), and the small signals from the propionate sidechain are seen for the first time. Analysis of other compounds shows the general feature that the methylene nearest the porphyrin periphery in this sidechain appears as a pair of multiplets separated by 0.7δ. In contrast, similar methylene groups in other (non-bridged) propionates appear as simple triplets (compare the example at 4.6 δ in Figure 30).

In the double bridged compound (73), whose n.m.r. spectrum is presented in Figure 32, there are no obscuring signals in the range 4 to 5 δ, and the multiplets for the methylene protons in question are clear. J. C. Waterton has examined this portion of the spectrum of similar compounds in detail at 270 MHz and has shown [106] that the observed coupling constants are consistent

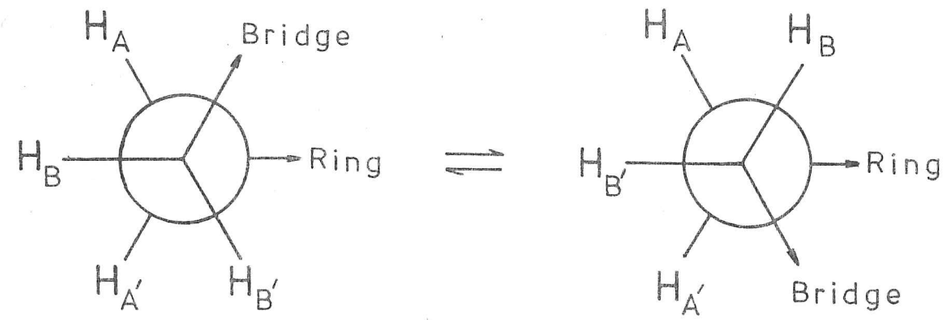


Bridge plus Tail

(57)

Figure 34

with fast exchange between the two conformations depicted in Figure 33:



Plan View

Figure 33

Protons H_A and $H_{A'}$ are in different environments and have distinct chemical shifts. The protons H_B and $H_{B'}$ which give signals near 3.3 δ in all relevant spectra also show a pattern of coupling constants consistent with this analysis [106].

The power of the relaxation method is apparent when it is applied to aid assignment of signals in complex spectra. The bridge plus tail compound (57) is a good example. The molecule contains 71 protons and the spectrum is presented in Figure 34. Chemical shift arguments are sufficient to assign many of the signals, but some details (given in the experimental section, Chapter 5) were determined by partial relaxation of an inverted spectrum to null out, for example, the residual protons of the pyridine solvent and allow observation of the otherwise hidden resonance at 7.54 δ assigned to a proton of the imidazole.

S. G. Hartley has shown that compounds like (57) with tails give special solvent shifts. These can be explained by noting that in solvents like chloroform the tail will tend to interact (via the imidazole) in a π - π aggregation with the porphyrin, and hence its protons will have their

resonances displaced upfield. In aromatic solvents like pyridine, the solvent competes effectively for the porphyrin, and the tail is directed on average away from the macrocycle, so its signals appear at more "normal" chemical shifts [113].

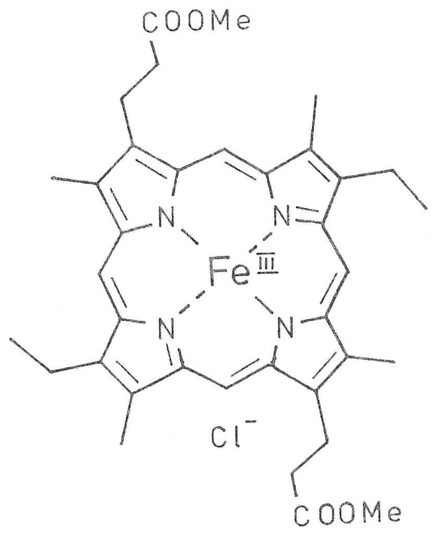
d) The proton n.m.r. of iron porphyrins

The n.m.r. spectra of two classes of iron porphyrins were of interest. One is formed by the paramagnetic low-spin ($S=\frac{1}{2}$) ferric complexes that result when a strong-field ligand (e.g. cyanide) is present in solution with the metalloporphyrin. The ligand-field splitting between the e_g and t_{2g} sets of orbitals in this d_5 system becomes so large that the electrons spin-pair as far as possible, leaving just one unpaired electron in one of the t_{2g} orbitals.

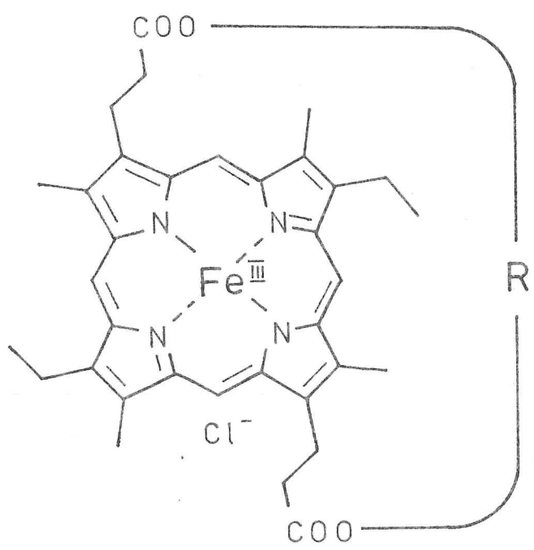
Addition of one further electron (to give the d_6 ferrous complex) allows complete spin-pairing in the presence of strong-field ligands and gives the diamagnetic Fe(II) form, which is the second type of compound studied.

The n.m.r. of iron porphyrins in other oxidation states or spin states has been reviewed [100], but the results are more difficult to interpret and provide less information about the structures.

K. Wüthrich et al. [114] showed that in low-spin ferric porphyrins very large chemical shift differences may be observed between signals that in diamagnetic or metal-free compounds are hardly resolved. For example, the four distinct ring methyl groups in protoporphyrin IX span a range of 5 ppm (from 12 δ to 17 δ) when the Fe(III) complex is examined, but less than 0.2 ppm in the corresponding metal-free material. The large downfield shift of the resonances (from 3.5 δ) is a consequence of hyperfine electron-nuclear interactions, which have two components. A contact effect operates through chemical bonds: some of the spin-density of the unpaired electron is delocalised on to the nucleus under observation. A pseudo- contact shift



(16a)



(25) $-R- = -(CH_2)_4C\equiv C-C\equiv C-(CH_2)_4-$

(26) $-R- = -(CH_2)_{12}-$

results from dipolar interactions between the electron spin and the nuclear spin (but is only observed if the magnetic field produced by the unpaired electron does not average to zero) [98, 102].

The shift caused by the contact term is a very sensitive function of the π-electron density in the HOMO of the macrocycle adjacent to the methyl groups, and therefore the methyl groups in the unsymmetrical molecule display widely varied chemical shifts.

The proton n.m.r. of Fe(III) mesoporphyrin II dimethyl ester (16a) was recorded in dimethylsulphoxide-d_6 containing potassium cyanide, under conditions in which the compound has spin $\frac{1}{2}$. The spectrum displayed a large range of chemical shifts, with the aromatic methyl groups at 11.95 and 14.20δ and the meso protons at 0.23 and 0.82δ; the general features following those observed before in related compounds (see Chapter 5 for full assignment) [114].

It would have been interesting to examine the bridged porphyrins under these conditions, since the effects on the resonance positions of the protons of the bridge must arise solely from pseudo-contact terms. The through-bond contact term will be diluted to zero out on the bridge, whereas the through-space pseudo-contact term (with a dependence on r^{-3} with distance r) would still operate. Unfortunately, when the available ferric complexes (25) or (26) were put in dimethylsulphoxide-d_6 containing potassium cyanide, very complex spectra resulted. It appears that the bridge hinders coordination of the ligand and that full six-coordination does not occur. The system is an equilibrium between a spin-$\frac{1}{2}$ and spin-$\frac{3}{2}$ or spin-$\frac{5}{2}$ states. As a result, a multiplicity of signals is observed and a simple spectral interpretation is not possible. For example, the ring methyl groups in (26) now give at least ten lines of varying intensity.

J. C. Waterton has demonstrated the effects of pseudo-contact shifts on the resonances of the protons of the bridge in the radical cation of the magnesium complex corresponding to (26). The unpaired spin-density causes a downfield shift, in opposition to that of the ring current, but with a

similar geometric dependence [106].

Diamagnetic iron porphyrins provide readily-interpretable n.m.r. spectra. Again the bridged porphyrins were of interest, as n.m.r. might provide structural insights not available in the paramagnetic molecules. Fe(II) porphyrins are quite stable in neat pyridine, even in the presence of oxygen [19], and the experimental procedure was simple. Anhydrous hydrazine was used to reduce the Fe(III) complexes. This was done in the Schlenk-tube equipment described in Chapter 5, section (e). Typically, 5 mg of metalloporphyrin in $\frac{1}{2}$ ml of pyridine was reduced under a nitrogen atmosphere with 20 μl hydrazine, in a flask attached through a side-arm to a supply of nitrogen and vacuum. The excess reagent (and solvent) were then removed by evaporation, whereupon deuteriopyridine was added from a syringe, through a rubber septum, and the ferrous porphyrin solution transferred to an n.m.r. tube.

The spectra of all diamagnetic ferroporphyrins were unsurprising. The signals appeared in similar sequence to those in the metal-free porphyrins, although over a narrower chemical shift range. Typically, the meso protons came at 9.9 δ (rather than 10.3 to 10.6 δ), and the bridge protons at 0.2 to 1.6 δ (rather than -1 to 1 δ). This result is in accord with W. S. Caughey's early observation that the ring current was diminished in metalloporphyrins with divalent transition metal ions [115], although metal complex formation may in some cases enhance the ring current [100].

CHAPTER FIVE EXPERIMENTAL

General Directions

Preparative layer chromatography was carried out using 20 x 20 cm glass plates coated with Merck Kieselgel 60 PF_{254} silica (1 mm thickness). Column chromatography was carried out with Fluka neutral alumina, type 507C, activity grade 3 on Brockmann scale (6% w/w water), or with Merck Kieselgel 60 silica, 70-230 mesh.

Melting points (m.p.) were determined on a Riechert-Kofler micro hot-stage apparatus, and are uncorrected.

Ultra-violet and visible spectra were recorded on a Unicam SP8000 spectrophotometer. The solvent used was chloroform for porphyrins and 95% ethanol for other compounds, unless otherwise stated.

Infra-red spectra were recorded on a Perkin-Elmer 257 spectrometer, in thin films (for liquids) or potassium bromide discs (for solids).

N.m.r. spectra were recorded (for non-porphyrins) in deuteriochloroform containing T.M.S., on a Varian HA-100, unless otherwise stated. Porphyrin n.m.r. were recorded on a Varian CFT-20 or XL-100, in deuteriopyridine. Those for metal-free porphyrins quoted in this chapter were taken on the 100 MHz instrument, for 0.013 M solutions at a probe temperature of 28°, with the following Fourier transform spectral parameters: spectral width 1536 Hz, acquisition time 2.666 sec, pulse width 70 μsec, sensitivity enhancement 1.3 sec, data length 8191, sweep offset 44440 Hz.

Mass spectra were recorded using A. E. I. MS12, MS30, or MS902 instruments, the latter for high resolution work.

Units and Conventions

All temperatures in degrees centigrade.

Infra-red absorption maxima $\nu_{max.}$ in wave numbers (cm^{-1}).

Ultra-violet and visible absorption maxima $\lambda_{max.}$ in nanometers (nm), and intensity ϵ ($1000\ cm^2\ mol^{-1}$) as $\log \epsilon$.

N.m.r. chemical shifts are given as δ values (ppm) from T.M.S. at zero, or d_4-pyridine at 8.700δ . The multiplicities of the signals are indicated by the abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Coupling constants (J) are given as J= ∝Hz.

Mass spectral peaks are quoted as m/e values, with molecular ion designated (M+), and relative intensities as % values of the molecular ion.

Purification of solvents

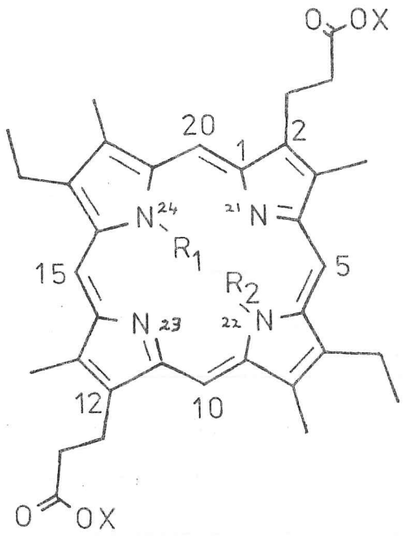
Solvents were purified when required, using published procedures [116]. Specific commonly used methods included:

Dichloromethane and dichloroethane were distilled from phosphorous pentoxide.

Tetrahydrofuran was distilled from lithium aluminium hydride and stored for short periods over sodium wire.

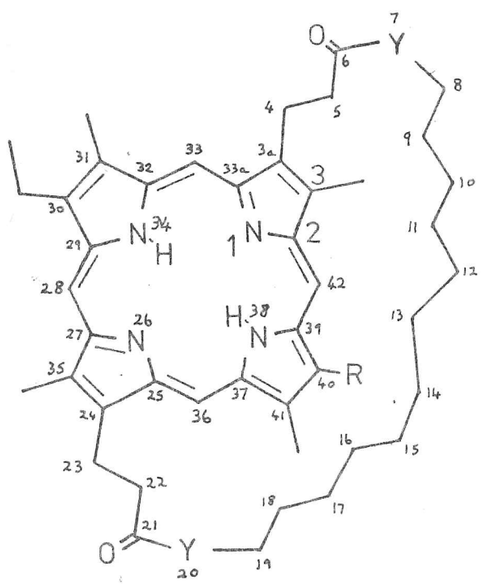
Pyridine was distilled from potassium hydroxide and stored at 0°.

Dimethylformamide for use as catalyst in the formation of acid chlorides was passed through a column of grade 1 alumina prior to use.



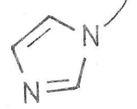
(16a) X = Me, R_1-R_2 = $-Fe^{III}(Cl)-$

(19) $X = R_1 = R_2 = H$



(23) Y = O, R = Et

(57) Y = $-\overset{H}{N}-$,

R = $(CH_2)_2CO\overset{H}{N}(CH_2)_3$ 

Note on the naming of compounds

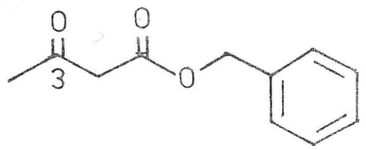
All non-porphyrins are named in this chapter according to current "Chemical Abstracts" conventions [117], even when they have been otherwise referred to in previous chapters. Porphyrins also follow Chemical Abstracts nomenclature when designated as "porphine" derivatives. However, at some points, trivial names have been used to save space or increase clarity. Bridged porphyrins in particular have clumsy systematic names. The examples below (with numbering shown opposite) show the systematic name for a representative sample of trivially-named compounds.

1) Mesoporphyrin II (19) : 7,17-Diethyl-3,8,17,18-tetramethyl-21H,23H-porphine-2,12-dipropanoic acid.

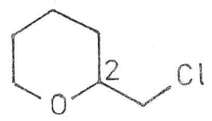
2) Mesoporphyrin II, dimethyl ester, iron (III) complex, chloride (16a): [Dimethyl 7,17-Diethyl-3,8,13,18-tetramethyl-21H,23H-porphine-2,12-dipropanoato(2-)-N^{21},N^{22},N^{23},N^{24}]-Iron(1+), chloride.

3) Mesoporphyrin II, dodeca-1,12-diol, cyclic diester (23): 30,40-Diethyl-4,5,7,8,9,10,11,12,13,14,15,16,17,18,19,20,22,23-octadecahydro-3,31,35,41-tetramethyl-38H-29,32-Imino-27,24-metheno-2,25-(metheno[2,5]-endo-pyrrolometheno)-2H-pyrrolo[2,3-o][7]aza[1,20]dioxacyclodotriacontine-6,21-dione.

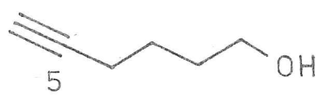
4) Bridge plus tail porphyrin (57): 30-Ethyl-4,5,7,8,9,10,11,12,13,14,15,16,17,18,19,20,22,23-octadecahydro-40-[3-[[3-(1H-imidazol-1-yl)propyl]amino]-3-oxopropyl]-3,31,35,41-tetramethyl-38H-29,32-Imino-27,24-metheno-2,25-(metheno[2,5]-endo-pyrrolometheno)-2H-pyrrolo[2,3-o][1,7,20]triazacyclodotriacontine-6,21-dione.



(1)



(17)



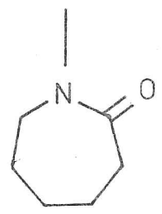
(18)

a) Miscellaneous compounds

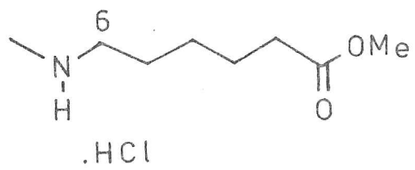
Phenylmethyl 3-Oxo-butanoate (1).- Benzyl alcohol (432 g, 4 M) and ethyl 3-oxo-butanoate (520 g, 4 M) were heated together in a flask fitted with a nitrogen inlet, thermometer, and Vigreux column. Distillate was collected from 78 to 85°, while the pot temperature rose to 210° (156 g were collected). The mixture was allowed to cool and was then vacuum distilled. After a forerun (b.p. 120-130° / 15 mm Hg), consisting of a mixture of starting materials, the product was obtained (551 g). The forerun was returned to the reaction flask and the procedure repeated to give a second crop of ester (119 g). Overall yield 670 g (87.2%), b.p. 158-162° / 15 mm Hg (lit., [118] 162-164° / 16 mm Hg), $\nu_{max.}$ 1 750 and 1 720 cm^{-1}; δ 2.2 (3H, s, CH_3CO), 3.5 (2H, s, $COCH_2COO$), 5.2 (2H, s, $PhCH_2$), 7.5 (5H, m, ArH).

2-Chloromethyl tetrahydropyran (17).- Thionyl chloride (62 g, 0.52 M) was added dropwise to tetrahydropyran-2-methanol (50 g, 0.43 M) in pyridine (80 ml), at such a rate as to maintain 43-48°, with vigorous stirring. After a further 5 h at 45°, the mixture was cooled and extracted with ether. The organic layer was washed with water, 0.5 N sulphuric acid, sodium hydrogen carbonate, and brine. Evaporation gave an oil which was vacuum distilled. The product (40.2 g, 70%) had b.p. 64-66° / 20 mm Hg (lit., [72] 55-55.5° / 6 mm Hg), δ 1.2 to 2.0 (6H, m, CH_2), 3.5 br (4H, m, CH_2O and CH_2Cl), 4.0 br (1H, m, 2-H).

Hex-5-yn-1-ol (18).- Sodium (25.9 g, 1.1 M) was cautiously added to liquid ammonia (450 ml) containing ferric nitrate nonahydrate (0.3 g), and stirred at reflux under an acetone / dry ice cooled condenser. The initially blue solution was stirred until sodamide formation was complete (ca. 1 h), as judged by the colour change to grey. The foregoing chloride (17) (35 g, 0.26 M) was then added over 40 min, and the mixture stirred for 3 h.



(27)

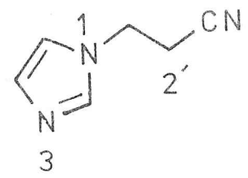


(28)

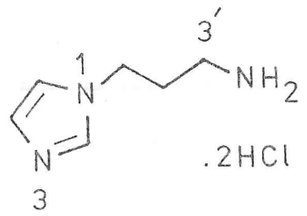
Ammonium chloride (60 g) was introduced over 20 min, and the whole allowed to warm overnight, evaporating the ammonia. The resultant mass was added to water (300 ml) and extracted with ether. The organic layer was washed with 2 N sulphuric acid, water, and brine, and on evaporation the residue was vacuum distilled. The acetylenic alcohol (20.7 g, 81%) had b.p. 81-82° / 18 mm Hg (lit., [72] 53° / 1.2 mm Hg), $\nu_{max.}$ 3 300 and 2 105 cm^{-1}; δ 1.6 br (4H, m, CH_2CH_2), 2.0 (1H, t, J 2 Hz, C≡CH), 2.2 br (2H, m, $CH_2C{\equiv}C$), 3.6 (2H, m, OCH_2), 3.8 (1H, OH).

N-methyl caprolactam (27).- Dimethyl sulphate (73.7 g, 0.585 M) was added dropwise to ∈-caprolactam (50 g, 0.442 M) in refluxing benzene (180 ml). Heating at reflux was continued for 18 h. The cooled organic layer was then washed with aqueous potassium carbonate until carbon dioxide evolution ceased, and then with water and brine. On evaporation and vacuum distillation, O-methyl caprolactam (b.p. 62-64° / 18 mm Hg) was the major product. This was heated at reflux in a nitrogen atmosphere for 3 h with dimethyl sulphate (0.2 ml) to rearrange it to the N-methyl compound. The oil that resulted was combined with the residue from the previous distillation and vacuum distilled to give product (26.9 g, 47.8%), b.p. 68-70° / 0.7 mm Hg (lit., [76] 120° / 19 mm Hg), $\nu_{max.}$ 1 700 cm^{-1}; δ 1.65 br (6H, m, CH_2), 2.4 (2H, m, CH_2CO), 2.86 (3H, s, N-Me), 3.4 (2H, m, CH_2N).

Methyl 6-Methylamino-hexanoate, hydrochloride (28).- N-methyl caprolactam (27) (10 g, 0.079 M) was stirred at reflux in boiling water (60 ml) and conc. hydrochloric acid (20 ml) for 1½ h. The solution was cooled and the solvent evaporated to give a clear gum which was stirred in a mixture of methanol (50 ml), trimethylorthoformate (50 ml) and conc. hydrochloric acid (0.5 ml) at 60°. Some material was allowed to distil away, and after 30 min the cooled solution was evaporated and the resultant syrup crystallised from methanol / ether containing 2,2-dimethoxy-propane. The salt (4.5 g, 29%) had m.p.



(53)

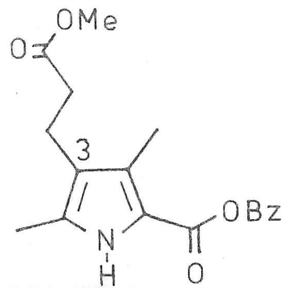


(54)

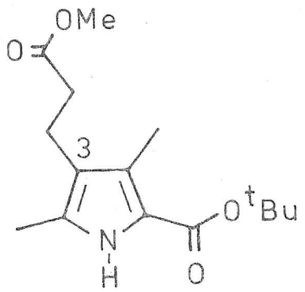
99-102° (Found: C, 49.24; H, 9.21; N, 7.32. $C_8H_{17}NO_2$.HCl requires C, 49.12; H, 9.27; N, 7.16%), $\nu_{max.}$ 1 725 cm^{-1}; δ 1.5 to 2.0 (6H, m, CH_2), 2.25 (2H, m, CH_2COO), 2.65 (3H, s, N-Me), 2.9 (2H, m, CH_2N), 3.6 (3H, s, COOMe).

1-(2-Cyanoethyl)-imidazole (53).- Acrylonitrile (53g, 1 M) was warmed to 75° and imidazole (34 g, 0.5 M) added to the flask down an attached reflux condenser over 20 min, with stirring. The temperature was raised to 95° and stirring continued for 3 h. On cooling and evaporating the excess acrylonitrile, the residue was vacuum distilled to afford 55.2 g (91%), b.p. 142-143° / 0.2 mm Hg, $\nu_{max.}$ 2 260 cm^{-1}; δ 2.78 (2H, t, CH_2CN), 4.18 (2H, t, NCH_2), 7.0 (2H, s, 4,5-H), 7.5 (1H, s, 2-H).

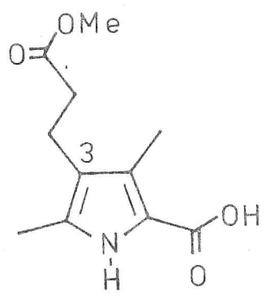
1-(3-Aminopropyl)-imidazole (54).- Raney nickel was prepared by digestion of Ni / Al alloy (12.5 g, 48% Ni) with sodium hydroxide (16 g) in water (60 ml) at 48-52°, followed by stirring at 50° for 1 h. The aqueous layer was removed by decantation and the nickel washed with water (4 x 100 ml) by decantation. It was then transferred to a centrifuge bottle in methanol and washed with methanol (4 x 100 ml). With care to avoid fire, the dry nickel was put in a Cook hydrogenator bottle with methanol (100 ml) and the mixture saturated with gaseous ammonia at 10°. The foregoing nitrile (53) (12.1 g, 0.1 M) was added in a little methanol and the whole hydrogenated at 50 p.s.i. for 18 h. The catalyst was removed by filtration and the solvent evaporated to yield the product as an oil (12.5 g, 100%). For analysis, it was treated in methanol with hydrogen chloride, and the salt so formed crystallised from ethanol, m.p. 147-148° (lit., [92] 149-150°) (Found: C, 36.64; H, 6.64; N, 20.93. $C_6H_{11}N_3$.2HCl requires C, 36.38; H, 6.61; N, 21.2%), δ ($(CD_3)_2SO$) 2.3 (2H, dt, J 6 Hz, $CH_2CH_2CH_2$), 2.82 (2H, t, J 6 Hz, 3'-CH_2), 4.48 (2H, t, J 6 Hz, 1'-CH_2), 7.68 and 7.91 (each 1H, d, J 1.5 Hz, 4,5-H), 9.4 (1H, s, 2-H), 8.52 (4H, NH).



(2a)



(2b)



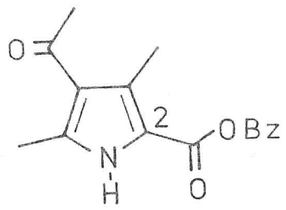
(3)

b) Pyrroles

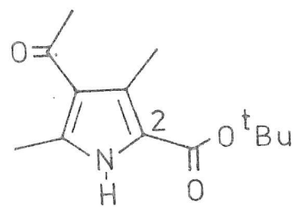
Methyl 2,4-Dimethyl-5-[(phenylmethoxy)carbonyl]-1H-pyrrole-3-propanoate (2a).- This was prepared from Phenylmethyl 3-Oxo-butanoate (1) and Methyl 4-Acetyl-5-oxo-hexanoate[119] by the method of A. W. Johnson *et al.*[63] (essentially as described for (4a) below) in 40% yield. m.p. 94-95° (lit.,[63] 99-100°)(Found: C, 68.42; H, 6.68; N, 4.31. $C_{18}H_{21}NO_4$ requires C, 68.55; H, 6.71; N, 4.44%), $\lambda_{max.}$ 283 nm; $\nu_{max.}$ 3 300, 1 735 and 1 665 cm^{-1}; δ 2.16 and 2.29 (each 3H, s, ring Me), 2.3 to 2.9 (4H, m, CH_2CH_2COO), 3.62 (3H, s, COOMe), 5.3 (2H, s, $PhCH_2$), 7.35 br (5H, m, ArH), 9.15 br (1H, s, NH).

Methyl 5-[(1,1-Dimethylethoxy)carbonyl]-2,4-dimethyl-1H-pyrrole-3-propanoate (2b).- This was prepared from 1,1-Dimethylethyl 3-Oxo-butanoate and Methyl 4-Acetyl-5-oxo-hexanoate[119] by the method of A. W. Johnson *et al.* (essentially as described for (4a) below) in 38% yield. m.p. 99-100° (lit.,[120] 69°)(Found: C, 63.82; H, 8.32; N, 4.89. $C_{15}H_{23}NO_4$ requires C, 64.03; H, 8.24; N, 4.98%), $\lambda_{max.}$ 281 nm; $\nu_{max.}$ 3 300, 1 730, and 1 660 cm^{-1}; δ 1.52 (9H, s, ^{t}Bu), 2.15 and 2.2 (each 3H, s, ring Me), 2.3 to 2.7 (4H, m, CH_2CH_2COO), 3.55 (3H, s, COOMe), 9.3 br (1H, s, NH).

Methyl 5-Carboxy-2,4-dimethyl-1H-pyrrole-3-propanoate (3).- A solution of the above pyrrole ester (2a)(25 g, 0.08 M) in dry tetrahydrofuran (200 ml) containing triethylamine (2 drops) was stirred at 20° with 10% palladised charcoal (0.5 g) and hydrogen for 3 h (uptake had then ceased). The catalyst was removed by filtration and the solution evaporated to afford the pyrrole carboxylic acid (17.8 g, 99%). An analytical sample crystallised from ethyl acetate had m.p. 135-137° (lit.,[121] 131°)(Found: C, 58.76; H, 6.93; N, 6.18. $C_{11}H_{15}NO_4$ requires C, 58.65; H, 6.71; N, 6.22%), $\lambda_{max.}$ 277 nm; $\nu_{max.}$ 3 300, 1 720, and 1 640 cm^{-1}; δ 2.2 and 2.28 (each 3H, s, ring Me), 2.4 to 2.8



(4a)



(4b)

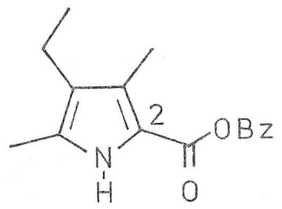
(4H, m, CH_2CH_2COO), 3.64 (3H, s, COOMe), 8.26 and 12 br (each 1H, s, NH, COOH).

Phenylmethyl 4-Acetyl-3,5-dimethyl-1H-pyrrole-2-carboxylate (4a).-

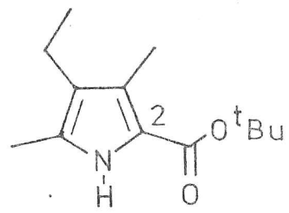
To a stirred solution of Phenylmethyl 3-Oxo-butanoate (1) (576 g, 3 M) in acetic acid (750 ml) was added a solution of sodium nitrite (225 g, 3.3 M) in water (300 ml), cooling to maintain 10 - 20°. When complete, the mixture was stirred at 25° for 1 h. Meanwhile, sodium carbonate (160 g, 1.5 M) was dissolved in acetic acid (1 l) in a 5 l flask and acetyl acetone (300 g, 3 M) added. Portions of the foregoing oxime solution were then added, while zinc dust (490 g, 7.5 M) was simultaneously mixed in to maintain 52 - 58°. Finally, the mixture was heated to 85° for 2 h and after decanting it into a large beaker, was allowed to cool overnight. The crude product which crystallised was isolated and washed well with water. It was then dissolved in dichloromethane (4 l), filtered free of zinc residues and separated from an aqueous layer before crystallisation was induced from the boiling mixture by the addition of hexane, at a total volume of 3 l. Further crops of product were taken from methanol. The pyrrole ester (497 g, 61.1%) had m.p. 134.5-135.5° (lit., [63] 135°)(Found: C, 70.92; H, 6.35; N, 5.31. $C_{16}H_{17}NO_3$ requires C, 70.83; H, 6.32; N, 5.16%), $\lambda_{max.}$ 285 nm; $\nu_{max.}$ 3 200, 1 680, and 1 630 cm^{-1}; δ 2.41 and 2.48 (each 3H, s, ring Me), 2.6 (3H, s, $COCH_3$), 5.31 (2H, s, $PhCH_2$), 7.35 (5H, m, ArH), 9.6 br (1H, s, NH).

1,1-Dimethylethyl 4-Acetyl-3,5-dimethyl-1H-pyrrole-2-carboxylate (4b).-

1,1-Dimethylethyl 3-Oxo-butanoate was converted essentially as described above for (4a) to give the pyrrole ester (80%), m.p. 147-149° (lit., [122] 148°) (Found: C, 65.85; H, 8.21; N, 5.92. $C_{13}H_{19}NO_3$ requires C, 65.80; H, 8.07; N, 5.90%), $\lambda_{max.}$ 284 nm; $\nu_{max.}$ 3 300 and 1 650 cm^{-1}; δ 1.05 (3H, t, J 8 Hz, Et CH_3), 1.6 (9H, s, ^tBu), 2.2 and 2.3 (each 3H, s, ring Me), 2.25 (2H, q, J 8 Hz, Et CH_2), 9.4 br (1H, s, NH).



(5a)



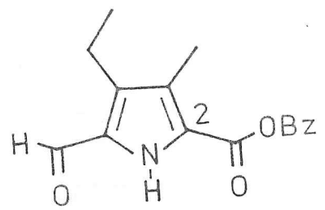
(5b)

Phenylmethyl 4-Ethyl-3,5-dimethyl-1H-pyrrole-2-carboxylate (5a).-

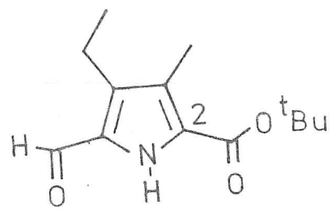
To a stirred solution under a closed nitrogen atmosphere of the foregoing acetyl pyrrole (4a) (226 g, 0.834 M) in dry tetrahydrofuran (1.5 l) containing sodium borohydride (50.7 g, 1.33 M) was added boron trifluoride etherate (200 ml, 1.6 M), cooling to maintain 0 - 2°. After stirring for a further 1 h, acetic acid was cautiously added until hydrogen evolution ceased. Water (50 ml) was added and the mixture allowed to warm up. The organic layer was filtered and washed with aqueous sodium carbonate and brine. On evaporation, the residue was taken in dichloromethane (500 ml) and dried over magnesium sulphate. Crystallisation from the boiling solution was induced by the slow addition of hexane (500 ml) at a total volume of 600 ml. Later crops were taken from ethanol and the pyrrole ester (195 g, 91%) had m.p. 102-104° (lit., [63] 103°)(Found: C, 74.61; H, 7.45; N, 5.72. $C_{16}H_{19}NO_2$ requires C, 74.67; H, 7.44; N, 5.44%), $\lambda_{max.}$ 287 nm; $\nu_{max.}$ 3 300 and 1 660 cm^{-1}; δ 1.04 (3H, t, J 8 Hz, Et CH_3), 2.15 and 2.28 (each 3H, s, ring Me), 2.37 (2H, q, J 8 Hz, Et CH_2), 5.28 (2H, s, $PhCH_2$), 7.35 (5H, m, ArH), 8.95 br (1H, s, NH).

1,1-Dimethylethyl 4-Ethyl-3,5-dimethyl-1H-pyrrole-2-carboxylate (5b).-

The corresponding pyrrole (4b) was treated essentially as described above for (5a) to yield the pyrrole ester (90%), m.p. 135.5-137° (lit., [120] 134-135°) (Found C, 69.96; H, 9.63; N, 6.19. $C_{13}H_{21}NO_2$ requires C, 69.92; H, 9.48; N, 6.27%), $\lambda_{max.}$ 283 nm; $\nu_{max.}$ 3 300 and 1650 cm^{-1}; δ 1.05 (3H, t, J 8 Hz, Et CH_3), 1.6 (9H, s, ^tBu), 2.2 and 2.3 (each 3H, s, ring Me), 2.25 (2H, q, J 8 Hz, Et CH_2), 9.4 br (1H, s, NH).



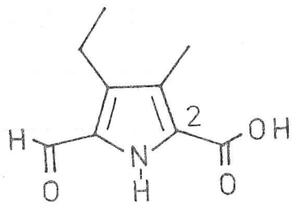
(6a)



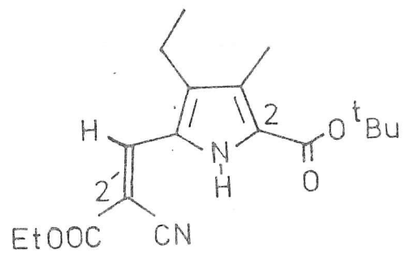
(6b)

Phenylmethyl 4-Ethyl-5-formyl-3-methyl-1H-pyrrole-2-carboxylate (6a).- To a stirred solution of the pyrrole ester (5a) (64.25 g, 0.25 M) in dichloromethane (700 ml) was added sulphuryl chloride (67.5 g, 0.5 M) in dichloromethane (500 ml) over 3 h, cooling to maintain -2 to 2°. After stirring for further 1 h at 0°, the organic layer was washed with water and aqueous sodium hydrogen carbonate until the washings were alkaline. Evaporation gave an oil which was taken up in tetrahydrofuran (300 ml), water (300 ml) added, and the whole heated at reflux for 1 h. The cooled solution was extracted into ether, washed with aqueous sodium hydrogen carbonate and evaporated to a brown oil which solidified on standing. Crystallisation from cyclohexane afforded the pyrrole aldehyde (61.3 g, 90%), m.p. 86-87° (lit., [65] 86-87°)(Found: C, 70.88; H, 6.44; N, 4.90. $C_{16}H_{17}NO_3$ requires C, 70.83; H, 6.32; N, 5.16%), $\lambda_{max.}$ 305 nm; $\nu_{max.}$ 3 270, 1 705, and 1 665 cm^{-1}; δ 1.2 (3H, t, J 8 Hz, Et CH_3), 2.29 (3H, s, ring Me), 2.74 (2H, q, J 8 Hz, Et CH_2), 5.33 (2H, s, $PhCH_2$), 7.38 (5H, m, ArH), 9.5 br (1H, s, NH), 9.74 (1H, s, CHO).

1,1-Dimethylethyl 4-Ethyl-5-formyl-3-methyl-1H-pyrrole-2-carboxylate (6b).- Lead tetraacetate (495 g) was added over 2 min to a solution of the pyrrole ester (5b) (98.2 g, 0.44 M) in acetic acid (600 ml) containing acetic anhydride (45 ml) and stirred in an Erlenmeyer flask. The temperature rose spontaneously to 80 - 90° and it was kept between these limits for a further 2.5 h. Ethylene glycol (50 ml) was added to destroy any excess oxidant, and water added to make the volume up to 2.5 l. The product was extracted into ether and washed with saturated aqueous sodium carbonate and brine. Evaporation yielded crude product as a brown oil (93.9 g, 90%) which proved difficult to crystallise. It may be used directly in the preparation of the adduct (8) as detailed below, for maximal overall yield. Alternatively, a first crop can be obtained from methanol / water in low yield and the remainder scavenged as (8). An analytical sample was sublimed at 50° and had



(7)

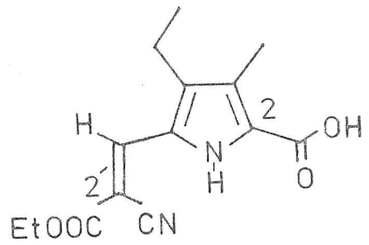


(8)

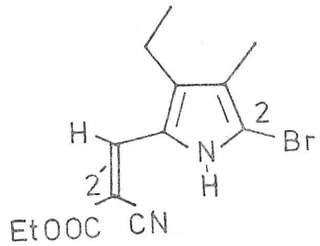
m.p. 63-66° (Found: C, 65.60; H, 8.08; N, 5.80. $C_{13}H_{19}NO_3$ requires C, 65.80; H, 8.07; N, 5.90%), $\lambda_{max.}$ 306 nm; $\nu_{max.}$ 3 200, 1 690, and 1 650 cm^{-1}; δ 1.2 (3H, t, J 8 Hz, Et CH_3), 1.56 (9H, s, ^tBu), 2.26 (3H, s, ring Me), 2.7 (2H, q, J 8 Hz, Et CH_2), 9.4 (1H, s, NH), 9.75 (1H, s, CHO).

4-Ethyl-5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid (7).- The pyrrole aldehyde (6a) (57 g, 0.21 M) in dry tetrahydrofuran (250 ml) was slowly filtered through a bed of Raney nickel, prepared by digestion of Ni / Al alloy (15 g) with sodium hydroxide (19.5 g) in water (75 ml) at 70°, followed by drying of the nickel by treatment with methanol. The pyrrole solution, now free of any sulphur impurities, was stirred at 20° with 10% palladised charcoal (1 g) and hydrogen for 7 h (uptake had then ceased). The solution was filtered and evaporated to give the pyrrole carboxylic acid (35.1 g, 92%). A sample crystallised from n-butanol had m.p. 199-201° (lit.,[123] 196-197°) (Found: C, 59.75; H, 6.37; N, 7.63. $C_9H_{11}NO_3$ requires C, 59.66; H, 6.12; N, 7.73%), $\lambda_{max.}$ 307 nm; $\nu_{max.}$ 3 160, 1 670, and 1 650 cm^{-1}; δ 1.14 (3H, t, J 8 Hz, Et CH_3), 2.24 (3H, s, ring Me), 2.77 (2H, q, J 8 Hz, Et CH_2), 9.7 (1H, s, CHO), 10.0 br (1H, s, NH).

1,1-Dimethylethyl 5-(2-cyano-2-ethoxycarbonylvinyl)-4-ethyl-3-methyl-1H-pyrrole-2-carboxylate (8).- To the foregoing crude aldehyde (6b) (94 g) was added ethanol (200 ml), ethyl cyanoacetate (50 g) and ethanolic diethylamine (10 ml of a 25% solution). The mixture was warmed on a steam-bath for 15 min. The product which crystallised on cooling was isolated and recrystallised from ethanol to yield the protected pyrrole (110 g, 76% based on (5b)), m.p. 116-118° (Found: C, 64.74; H, 7.29; N, 8.40. $C_{18}H_{24}N_2O_4$ requires C, 65.03; H, 7.28; N, 8.43%), $\lambda_{max.}$ 380 nm; $\nu_{max.}$ 3 400, 2 195, 1 705, and 1 590 cm^{-1}; δ 1.18 (3H, t, J 8 Hz, Et CH_3), 1.42 (3H, t, J 7 Hz, ester Me), 1.63 (9H, s, ^tBu), 2.34 (3H, s, ring Me), 2.67 (2H, q, J 8 Hz, Et CH_2), 4.40 (2H, q, J 7 Hz, ester CH_2), 8.05 (1H, s, vinylH), 10.2 (1H, s, NH).



(9)

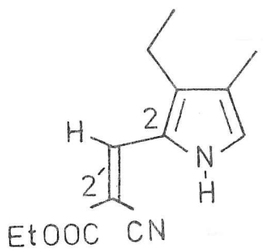


(10)

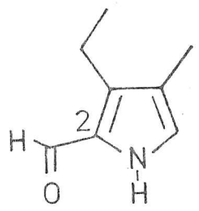
5-(2-Cyano-2-ethoxycarbonylvinyl)-4-ethyl-3-methyl-1H-pyrrole-2-carboxylic acid (9).- A solution of the above pyrrole ester (8) (76 g, 0.23 M) in 1,2-dichloroethane (250 ml) was heated at reflux under nitrogen and trifluoroacetic acid (30 ml) added. After 4 h, the mixture was cooled and the product recovered by filtration. Further quantities were induced to crystallise by the addition of hexane and these were recrystallised from ethyl acetate to afford the pyrrole carboxylic acid (53 g, 83%), m.p. 210-213° (Found: C, 60.86; H, 6.00; N, 10.14. $C_{14}H_{16}N_2O_4$ requires C, 60.86; H, 5.84; N, 10.14%), $\lambda_{max.}$ 387 nm; $\nu_{max.}$ 3 400, 2 200, 1 730, 1 650, and 1 585 cm^{-1}; δ 1.17 (3H, t, J 8 Hz, Et CH_3), 1.42 (3H, t, J 8 Hz, ester Me), 2.35 (3H, s, ring Me), 2.62 (2H, q, J 8 Hz, Et CH_2), 4.4 (2H, q, J 8 Hz, ester CH_2), 8.15 (1H, s, vinylH), 10.15 br (1H, s, NH).

2-Bromo-5-(2-cyano-2-ethoxycarbonylvinyl)-4-ethyl-3-methyl-1H-pyrrole (10).- Bromine (6.2 g, 0.039 M) in acetic acid (25 ml) was added dropwise at 70° to a solution of the preceding pyrrole carboxylic acid (9) (10.3 g, 0.038 M) in acetic acid (80 ml) containing sodium acetate (10 g). When the addition was complete, the solution was cooled and water (150 ml) added to complete the precipitation of the product, which was isolated, washed well with hexane and recrystallised from methanol to give the bromopyrrole (9 g, 76%), m.p. 105-108°, $\lambda_{max.}$ 396 nm; $\nu_{max.}$ 3 250, 2 205, and 1 710 cm^{-1}; δ 1.1 (3H, t, J 8 Hz, Et CH_3), 1.35 (3H, t, J 8 Hz, ester Me), 2.0 (3H, s, ring Me), 2.6 (2H, q, J 8 Hz, Et CH_2), 4.3 (2H, q, J 8 Hz, ester CH_2), 7.87 (1H, s, vinylH), 9.6 br (1H, s, NH).

2-(2-Cyano-2-ethoxycarbonylvinyl)-4-ethyl-3-methyl-1H-pyrrole (11).- A solution of the above bromopyrrole (10) (3.06 g, 9.8 mM) in dry tetrahydrofuran (75 ml) containing sodium acetate (3 g) was stirred at 20° with 10% palladised charcoal (0.3 g) and hydrogen for 20 min (uptake had then ceased). The filtered solution was evaporated and the residue crystallised from



(11)



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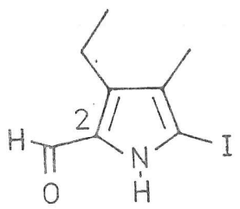


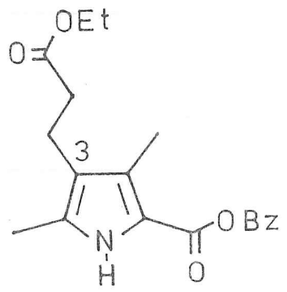
(13)

methanol to give the protected α-free pyrrole (1.63 g, 70%), m.p. 123-125° (Found: C, 66.96; H, 7.19; N, 12.17. $C_{13}H_{16}N_2O_2$ requires C, 67.22; H, 6.94; N, 12.06%), $\lambda_{max.}$ 388 nm; $\nu_{max.}$ 3 380, 2 195, and 1 685 cm^{-1}; δ 1.15 (3H, t, J 8 Hz, Et CH_3), 1.4 (3H, t, J 8 Hz, ester Me), 2.1 (3H, s, ring Me), 2.65 (2H, q, J 8 Hz, Et CH_2), 4.35 (2H, q, J 8 Hz, ester CH_2), 7.05 (1H, d, J 2 Hz, α-H), 8.15 (1H, s, vinylH), 9.45 br (1H, s, NH).

3-Ethyl-4-methyl-1H-pyrrole-2-carboxaldehyde (12).- The above protected pyrrole (11) (1.32 g, 5.7 mM) was heated at reflux under nitrogen with aqueous potassium hydroxide (25 ml of a 2M solution) for 1.5 h. On cooling, the product was extracted into ether and after evaporation and crystallisation from light petroleum (b.p. 60 - 80°) afforded the pyrrole aldehyde (0.63 g, 87%), m.p. 74-76° (lit.,[123] 49°)(Found: C, 70.04; H, 8.08; N, 10.21. $C_8H_{11}NO$ requires C, 70.08; H, 8.14; N, 10.26%), $\lambda_{max.}$ 301 nm; $\nu_{max.}$ 3 250 and 1 640 cm^{-1}; δ 1.2 (3H, t, J 8 Hz, Et CH_3), 2.02 (3H, s, ring Me), 2.72 (2H, q, J 8 Hz, Et CH_2), 6.86 (1H, d, J 2 Hz, α-H), 9.55 (1H, s, CHO), 10.3 br (1H, s, NH).

Alternatively, this pyrrole can be prepared from the iodo aldehyde (13) in low (ca. 40%) yield by hyrogenation in tetrahydrofuran containing magnesium oxide.

3-Ethyl-5-iodo-4-methyl-1H-pyrrole-2-carboxaldehyde (13).- The pyrrole aldehyde (7) (6.5 g, 0.036 M) was added to a mixture of water (50 ml), sodium hydrogen carbonate (9 g, 0.108 M), magnesium oxide (3 g), and chloroform (50 ml). While heating at reflux, iodine (10 g, 0.039 M) and sodium iodide (10 g) in water (50 ml) were added. After 30 min, sodium metabisulphite (1 g) was added to the cooled mixture to destroy excess iodine. The organic layer was separated and washed with water, before filtering through a bed of sodium sulphate to dry it. On evaporation, the red gum (8.5 g, 90%), which was pure by n.m.r., was crystallised from ether / hexane to give the iodo



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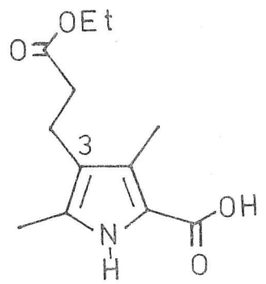


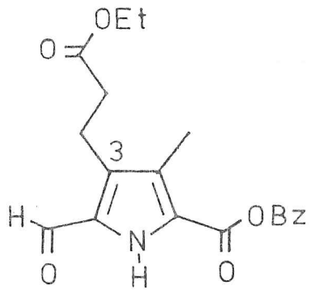
(38)

aldehyde (4.2 g, 44.5%), m.p. 114-115° (lit.,[123] 114°)(Found: C, 36.80; H, 3.87; N, 5.42. $C_8H_{10}NOI$ requires C, 36.52; H, 3.83; N, 5.32%), $\lambda_{max.}$ 316 nm; $\nu_{max.}$ 3 250, 1 640, and 1 620 cm^{-1}; δ 1.22 (3H, t, J 8 Hz, Et CH_3), 2.0 (3H, s, ring Me), 2.77 (2H, q, J 8 Hz, Et CH_2), 9.4 (1H, s, CHO), 10.1 br (1H, s, NH).

Ethyl 2,4-Dimethyl-5-[(phenylmethoxy)carbonyl]-1H-pyrrole-3-propanoate (37).- This was prepared from Phenylmethyl 3-Oxo-butanoate (1) and Ethyl 4-Acetyl-5-oxo-hexanoate[119] by the method of A. W. Johnson *et al.*[63] (essentially as described for (4a) above) in 43% yield. m.p. 73-75.5° (lit.,[124] 75-76°)(Found: C, 69.2; H, 7.02; N, 4.21. $C_{19}H_{23}NO_4$ requires C, 69.3; H, 7.05; N, 4.25%), $\lambda_{max.}$ 283 nm; $\nu_{max.}$ 3 310, 1 725, and 1 665 cm^{-1}; δ 1.24 (3H, t, J 7 Hz, Et CH_3), 2.20 and 2.31 (each 3H, s, ring Me), 2.3 to 2.9 (4H, m, CH_2CH_2COO), 4.11 (2H, q, J 7 Hz, Et CH_2), 5.3 (2H, s, $PhCH_2$), 7.35 br (5H, m, ArH), 9.2 br (1H, s, NH).

Ethyl 5-Carboxy-2,4-dimethyl-1H-pyrrole-3-propanoate (38).- The above ethyl ester (37) was hydrogenated essentially as described for the formation of (3) above, to give the carboxylic acid (95%), m.p. 124-126° (Found: C, 60.30; H, 7.15; N, 5.73. $C_{12}H_{17}NO_4$ requires C, 60.23; H, 7.16; N, 5.85%), $\lambda_{max.}$ 277 nm; $\nu_{max.}$ 3 300, 1 710, and 1 640 cm^{-1}; δ 1.25 (3H, t, J 7 Hz, Et CH_3), 2.24 and 2.32 (each 3H, s, ring Me), 2.4 to 2.7 (4H, m, CH_2CH_2COO), 4.12 (2H, q, J 7 Hz, Et CH_2), 8.86 br (1H, s, NH).

Ethyl 2-Formyl-4-methyl-5-[(phenylmethoxy)carbonyl]-1H-pyrrole-3-propanoate (41).- The pyrrole ester (37) (50 g, 0.152 M) in dichloromethane (500 ml) was treated with sulphuryl chloride (41 g, 0.304 M), essentially as described for the formation of (6a) above. After crystallisation from carbon tetrachloride / light petroleum (b.p. 60-80°), 39.2 g (75.2%) of the aldehyde was obtained. Further material could be scavenged as the ketal (46) (see below).



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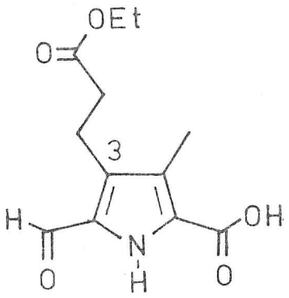

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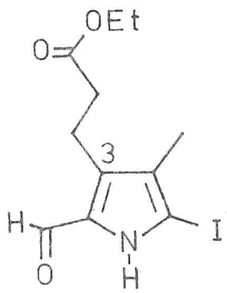
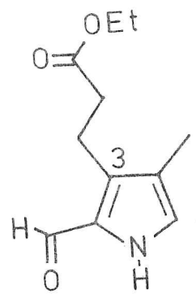


(43)

The product had m.p. 81-83.5° (Found: C, 66.18; H, 6.02; N, 3.83. $C_{19}H_{21}NO_5$ requires C, 66.46; H, 6.17; N, 4.08%), $\lambda_{max.}$ 305 nm; $\nu_{max.}$ 3 290, 1 745, 1 710, and 1 675 cm^{-1}; δ 1.2 (3H, t, J 7 Hz, Et CH_3), 2.30 (3H, s, ring Me), 2.53 (2H, t, J 7 Hz, CH_2COO), 3.04 (2H, t, J 7 Hz, $\underline{CH}_2CH_2COO$), 4.08 (2H, q, J 8 Hz, Et CH_2), 5.32 (2H, s, $PhCH_2$), 7.38 br (5H, m, ArH), 9.75 br (1H, s, NH), 9.79 (1H, s, CHO).

Ethyl 5-Carboxy-2-formyl-4-methyl-1H-pyrrole-3-propanoate (42).- The pyrrole aldehyde (41) (70.6 g, 0.2 M) was hydrogenated as described above for the preparation of (7), and on evaporation of solvent gave 52 g (100%). An analytical sample was crystallised from carbon tetrachloride / ethyl acetate and had m.p. 150-152° (Found: C, 56.63; H, 5.91; N, 5.43. $C_{12}H_{15}NO_5$ requires C, 56.90; H, 5.97; N, 5.53%), $\lambda_{max.}$ 306 nm; $\nu_{max.}$ 3 270, 1 730, 1 705, and 1 670 cm^{-1}; δ 1.24 (3H, t, J 7 Hz, Et CH_3), 2.36 (3H, s, ring Me), 2.6 (2H, t, J 7 Hz, CH_2COO), 3.10 (2H, t, J 7 Hz, $\underline{CH}_2CH_2COO$), 4.12 (2H, q, J 7 Hz, Et CH_2), 9.84 (1H, s, CHO).

Ethyl 2-Formyl-5-iodo-4-methyl-1H-pyrrole-3-propanoate (43).- The above pyrrole acid (42) (5.4 g, 21 mM) in water (75 ml) and chloroform (100 ml) containing sodium hydrogen carbonate (5.4 g, 64 mM), was heated at reflux while iodine (5.42 g, 21 mM) and sodium iodide (6 g) in water (150 ml) were added. After a further 15 min, sodium metabisulphite (1 g) was added to the cooled solution to destroy excess iodine. The organic layer was separated and washed with water. On evaporation, the iodo-aldehyde crystallised. It was recrystallised from ether / light petroleum (b.p. 60-80°) to yield 5.37 g (75%), m.p. 70-72° (Found: C, 39.34; H, 4.33; N, 4.06. $C_{11}H_{14}NO_3I$ requires C, 39.42; H, 4.21; N, 4.18%), $\lambda_{max.}$ 315 nm; $\nu_{max.}$ 3 210, 1 730, and 1 630 cm^{-1}; δ 1.3 (3H, t, J 7 Hz, Et CH_3), 2.08 (3H, s, ring Me), 2.56 (2H, t, J 7 Hz, CH_2COO), 3.1 (2H, t, J 7 Hz, CH_2CH_2COO), 4.15 (2H, q, J 7 Hz, Et CH_2), 9.50 (1H, s, CHO).

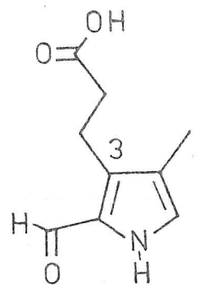


(44)

Ethyl 2-Formyl-4-methyl-1H-pyrrole-3-propanoate (44).- The iodo-aldehyde (43) (5.37 g, 16 mM) in methanol (100 ml) containing sodium acetate (5.4 g) was stirred at 20° with 10% palladised charcoal (0.2 g) and hydrogen. After several hours, the theoretical uptake had not occurred, so platinum oxide (50 mg) was added and hydrogenolysis continued to completion. The filtered solution was evaporated and the product extracted into ether, washing the organic layer with water. Evaporation and recrystallisation from ether / hexane afforded the α-free aldehyde (2.83 g, 84.5%), m.p. 66-69° (Found: M+, 209.1069. $C_{11}H_{15}NO_3$ requires 209.1052), $\lambda_{max.}$ 302 nm; $\nu_{max.}$ 3 250, 1 725, and 1 650 cm^{-1}; δ 1.22 (3H, t, J 7 Hz, Et CH_3), 2.06 (3H, s, ring Me), 2.48 (2H, t, J 7 Hz, CH_2COO), 3.0 (2H, t, J 7 Hz, CH_2CH_2COO), 6.86 br (1H, d, α-H), 9.54 (1H, s, CHO), m/e 209 (M+).

The above compound may also be produced in low yield from the protected aldehyde (47) by the following procedure:

The acid (47) (4.23 g, 12.5 mM) was iodinated following the method given above for the unprotected aldehyde (43). The crude iodo compound was not characterised, but taken immediately in a hydrogenolysis, following that for (44) above, using platinum oxide (50 mg) as catalyst. The α-free protected aldehyde which resulted after extraction into dichloromethane was evidently unstable, as judged by its increasing redness with time, so it was at once deprotected by treatment in ethanol (75 ml) with a mixture of trifluoroacetic acid (15 ml) and water (5 ml) at 20° for 15 min. The reaction mixture was poured into dichloromethane (100 ml) and water (100 ml), and the acid neutralised by cautious addition of solid sodium hydrogen carbonate. The organic layer was separated and washed with water before evaporating to an oil, which, after chromatography on Merck silica (50 g) eluting with dichloromethane, afforded the aldehyde (108 mg, 4.1% over 3 steps), after crystallisation.



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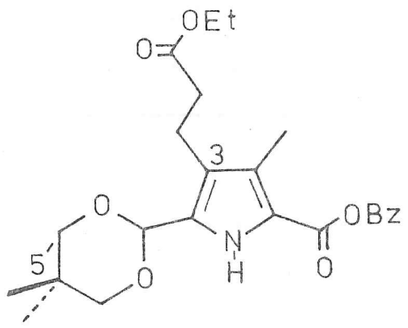
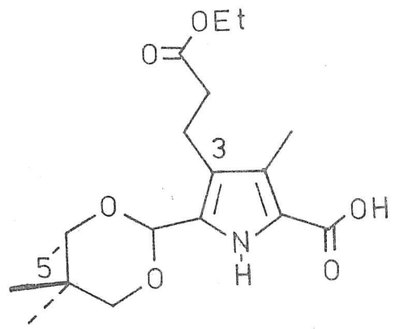


(46)

2-Formyl-4-methyl-1H-pyrrole-3-propanoic acid (45).- The foregoing pyrrole ester (44) (2.83 g, 13.5 mM) in dry ethanol (100 ml) containing potassium hydroxide (3 g, 54 mM) was heated at reflux under a nitrogen atmosphere for 2 h. The cooled solution was evaporated and the residue neutralised in water to pH 2 by the addition of conc. hydrochloric acid. The product was extracted into ethyl acetate and the organic layer dried over sodium sulphate before evaporation. Crystallisation from benzene / ethyl acetate / hexane gave the free acid (1.93 g, 78.7%), m.p. 121-123° (Found: C, 59.56; H, 6.28; N, 7.60. $C_9H_{11}NO_3$ requires C, 59.66; H, 6.12; N, 7.73%), $\lambda_{max.}$ 299 nm; $\nu_{max.}$ 3 200, 1 720, and 1 615 cm^{-1}; δ (CD_3OD) 2.04 (3H, s, ringMe), 2.52 (2H, t, J 7 Hz, CH_2COO), 3.04 (2H, t, J 7 Hz, CH_2CH_2COO), 6.98 (1H, s, α-H), 9.50 (1H, s, CHO).

Ethyl 2-[5,5-Dimethyl-1,3-dioxan-2-yl]-4-methyl-5-[(phenylmethoxy)carbonyl]-1H-pyrrole-3-propanoate (46).- The pyrrole aldehyde (41) (15 g, 43.7 mM), neopentyl glycol (45 g, 0.437 M), and p-toluene sulphonic acid (0.2 g) in benzene (100 ml) were heated at reflux for 2 h. On cooling, the organic layer was washed with aqueous sodium carbonate and water. The evaporated solution was filtered in ether through a bed of neutral alumina (100 g, grade 1), and the purified product crystallised from hexane to yield 8.83 g (47%), m.p. 87-90° (Found: C, 66.82; H, 7.34; N, 3.27. $C_{24}H_{31}NO_6$ requires C, 67.11; H, 7.28; N, 3.30%), $\lambda_{max.}$ 275 nm; $\nu_{max.}$ 3 260, 1 740, and 1 680 cm^{-1}; δ 0.74 and 1.22 (each 3H, s, 5'-Me), 1.2 (3H, t, J 7 Hz, Et CH_3), 2.24 (3H, s, ring Me), 2.32 (2H, m, CH_2COO), 2.68 (2H, m, CH_2CH_2COO), 3.61 (4H, dd, dioxan CH_2), 4.03 (2H, t, J 7 Hz, Et CH_2), 5.22 (2H, s, $PhCH_2$), 5.41 (1H, s, OCHO), 7.3 br (5H, m, ArH), 8.90 br (1H, s, NH).

5-Carboxy-2-[5,5-dimethyl-1,3-dioxan-2-yl]-4-methyl-1H-pyrrole-3-propionic acid (47).- The above pyrrole (46) (8.45 g, 19.7 mM) in tetrahydrofuran (100 ml) was stirred with 5% palladised charcoal (0.2 g) and hydrogen at 20° for 2.5 h (uptake had then ceased). The filtered solution was evaporated



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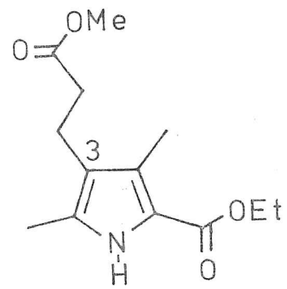

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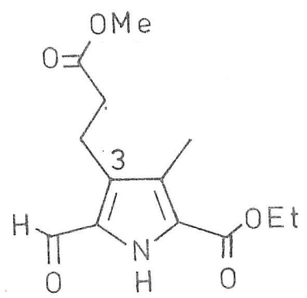
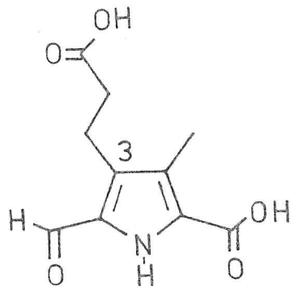


(50)

and on trituration with hexane gave the desired free acid (6.3 g, 94%). An analytical sample was recrystallised from heptane and a little tetrahydrofuran, m.p. 143-147° (Found: C, 60.31; H, 7.51; N, 4.03. $C_{17}H_{25}NO_6$ requires C, 60.16; H, 7.43; N, 4.13%), $\lambda_{max.}$ 266 nm; $\nu_{max.}$ 3 320, 1 710, and 1 660 cm^{-1}; δ 0.78 and 1.25 (each 3H, s, 5'-Me), 1.24 (3H, t, J 7 Hz, Et CH_3), 2.3 (3H, s, ring Me), 2.46 (2H, m, CH_2COO), 2.8 (2H, m, CH_2CH_2COO), 3.64 and 3.74 (each 2H, d, J 12 Hz, dioxan CH_2), 4.12 (2H, q, J 7 Hz, Et CH_2), 5.52 (1H, s, OCHO), 9.13 br (1H, s, NH).

Methyl 5-Ethoxycarbonyl-2,4-dimethyl-1H-pyrrole-3-propanoate (49).- This was prepared from Ethyl 3-Oxo-butanoate and Methyl 4-Acetyl-5-oxo-hexanoate [119] by the method of A. W. Johnson et al. [63] (essentially as described for (4a) above) in 47.7% yield. m.p. 100-102° (lit., [52] 104°) (Found: C, 61.56; H, 7.58; N, 5.57. $C_{13}H_{19}NO_4$ requires C, 61.64; H, 7.56; N, 5.53%), $\lambda_{max.}$ 282 nm; $\nu_{max.}$ 3 300, 1 730, and 1 665 cm^{-1}; δ 1.44 (3H, t, J 8 Hz, Et CH_3), 2.23 and 2.28 (each 3H, s, ring Me), 2.3 to 2.8 (4H, m, CH_2CH_2COO), 3.86 (3H, s, ester Me), 4.3 (2H, q, J 8 Hz, Et CH_2), 9.7 br (1H, s, NH).

Methyl 5-Ethoxycarbonyl-2-formyl-4-methyl-1H-pyrrole-3-propanoate (50).- The above pyrrole ester (49) (25.3 g, 0.1 M) in dichloromethane (300 ml) was treated with sulphuryl chloride (27 g, 0.2 M), essentially as described above for the preparation of (6a) and after crystallisation from carbon tetrachloride / ether / light petroleum (b.p. 60-80°) gave 17.1 g (64%), m.p. 71-74° (lit., [125] 141-143° ex. methanol / water) (Found: C, 58.28; H, 6.45; N, 5.22. $C_{13}H_{17}NO_5$ requires C, 58.41; H, 6.41; N, 5.24%), $\lambda_{max.}$ 303 nm; $\nu_{max.}$ 3 280, 1 715, 1 690, and 1 670 cm^{-1}; δ 1.36 (3H, t, J 7 Hz, Et CH_3), 2.3 (3H, s, ring Me), 2.56 (2H, t, J 7 Hz, CH_2COO), 3.05 (2H, t, J 7 Hz, CH_2CH_2COO), 3.64 (3H, s, ester Me), 4.35 (2H, q, J 7 Hz, Et CH_2), 9.65 br (1H, s, NH), 9.81 (1H, s, CHO).



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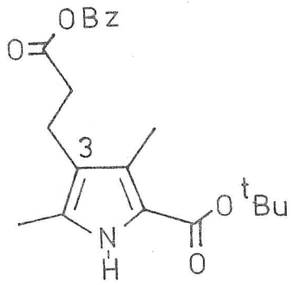

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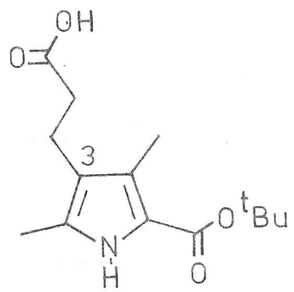


(59)

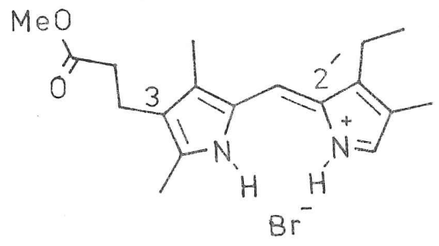
5-Carboxy-2-formyl-4-methyl-1H-pyrrole-3-propanoic acid (51).-

The pyrrole ester (50) (8.3 g, 31 mM) in dry ethanol (75 ml) containing potassium hydroxide (8 g) was heated at reflux in a nitrogen atmosphere for 2 h. The salt produced was isolated by filtration, dissolved in water (100 ml), and neutralised to pH 2 with conc. hydrochloric acid. The product was dried under high vacuum to yield 5.7 g (81%). An analytical sample crystallised from water / ethanol had m.p. 227-230° (lit.,[126] 230°) (Found: C, 53.48; H, 5.06; N, 6.00. $C_{10}H_{11}NO_5$ requires C, 53.33; H, 4.92; N, 6.22%), $\lambda_{max.}$ 307 nm; $\nu_{max.}$ 3 260, 1 710, 1 695, and 1 670 cm^{-1}; δ ($(CD_3)_2SO$) 2.24 (3H, s, ring Me), 2.42 (2H, t, J 7 Hz, CH_2COO), 2.94 (2H, t, J 7 Hz, CH_2CH_2COO), 9.76 (1H, s, CHO), 13.2 br (1H, s, NH).

Benzyl 5- (1,1-Dimethylethoxy)carbonyl -2,4-dimethyl-1H-pyrrole-3-propanoate (58).- The pyrrole ester (2b) (28.1 g, 0.1 M) in dry benzyl alcohol (28 ml) was heated to 85° and portions of a solution of sodium benzyloxide in benzyl alcohol (prepared from 0.5 g sodium in 15 ml alcohol) were added over 1 h, while dry nitrogen was blown over the stirred mixture. After cooling to 65°, the mixture was poured into methanol / water (150 ml of each) with stirring. The precipitated product was crystallised from iso-propanol to give 31.7 g (88.7%), m.p. 83-84° (Found: C, 70.28; H, 7.61; N, 3.65. $C_{21}H_{27}NO_4$ requires C, 70.56; H, 7.62; N, 3.92%), $\lambda_{max.}$ 280 nm; $\nu_{max.}$ 3 3300, 1 740, and 1 650 cm^{-1}; δ 1.54 (9H, s, ^tBu), 2.14 and 2.21 (each 3H, s, ring Me), 2.3 to 2.8 (4H, m, CH_2CH_2COO), 5.06 (2H, s, $PhCH_2$), 7.28 br (5H, m, ArH), 9.0 (1H, s, NH).

5- (1,1-Dimethylethoxy)carbonyl -2,4-dimethyl-1H-pyrrole-3-propionic acid (59).- The above pyrrole ester (58) (20 g, 56 mM) was hydrogenated as described above for (3), and on evaporation gave 15 g (100%). An analytical sample crystallised from chloroform / light petroleum (b.p. 60-80°) had m.p. 186-187° (Found: C, 63.0; H, 7.79; N, 5.38. $C_{14}H_{21}NO_4$ requires C, 62.9;

H, 7.92; N, 5.24%), $\lambda_{max.}$ 281 nm; $\nu_{max.}$ 3 340, 1 710, and 1 655 cm^{-1}; δ 1.56 (9H, s, ^{t}Bu), 2.18 and 2.26 (each 3H, s, ring Me), 2.5 to 2.7 (4H, m, CH_2CH_2COO), 9.5 br (1H, s, NH).



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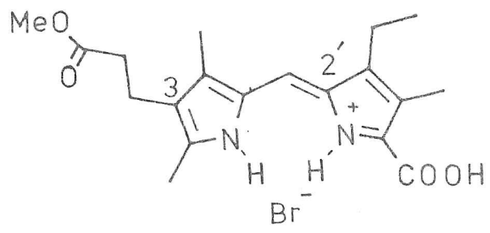


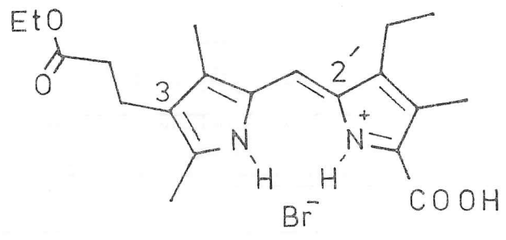
(15)

c) Dipyrromethenes

Methyl 5-[[3-Ethyl-4-methyl-2H-pyrrol-2-ylidene]methyl]-2,4-dimethyl-1H-pyrrole-3-propanoate, monohydrobromide (14).- To a solution of the pyrrole carboxylic acid (3) (9.7 g, 0.043 M) and the pyrrole aldehyde (12) (5.88 g, 0.043 M) in methanol (30 ml) stirred at 20° was added aqueous hydrobromic acid (10 ml of a 48% solution). The mixture was warmed to reflux for 15 min and then trimethylorthoformate (20 ml) was cautiously added to re-esterify the propionate side-chain. Cooling and evaporating, followed by crystallising from methanol / ether gave the dipyrromethene (13.1 g, 80%), m.p. 145-148° (Found: C, 56.54; H, 6.59; N, 7.13. $C_{18}H_{25}N_2O_2Br$ requires C, 56.69; H, 6.61; N, 7.35%), $\lambda_{max.}$ 481 nm (log ϵ 5.0); $\nu_{max.}$ 1 730 and 1 620 cm^{-1}; δ 1.2 (3H, t, J 8 Hz, Et CH_3), 2.08 (3H, s, 4'-Me), 2.36 (3H, s, 3-Me), 2.55 (2H, q, J 8 Hz, Et CH_2), 2.7 (4H, m, CH_2CH_2COO), 2.72 (3H, s, 5-Me), 3.67 (3H, s, OMe), 7.18 (1H, s, metheneH), 7.51 (1H, d, J 3 Hz, 5'-H), 13.2 br (2H, NH).

Methyl 5-[[5-Carboxy-3-ethyl-4-methyl-2H-pyrrol-2-ylidene]methyl]-2,4-dimethyl-1H-pyrrole-3-propanoate, monohydrobromide (15).- To a solution of the pyrrole carboxylic acid (3) (13.7 g, 0.061 M) and the pyrrole aldehyde (7) (11.02 g, 0.061 M) in acetic acid (50 ml) stirred at 20° was added hydrogen bromide in acetic acid (35 ml of a 48% solution). Stirring was continued for 1 h, the solvent evaporated and the residue crystallised from chloroform / ether to give the dipyrromethene (21.7 g, 84%), m.p. 137-140° (decomp.)(Found: C, 53.28; H, 5.88; N, 6.51. $C_{19}H_{25}N_2O_4Br$ requires C, 53.60; H, 5.92; N, 6.59%), $\lambda_{max.}$ 395 and 489 nm (log ϵ 4.88); $\nu_{max.}$ 1 740, 1 710, and 1 620 cm^{-1}; δ 1.15 (3H, t, J 7 Hz, Et CH_3), 2.22 (3H, s, 4'-Me), 2.4 (3H, s, 3-Me), 2.6 (2H, q, J 7 Hz, Et CH_2), 2.7 (4H, m, CH_2CH_2COO), 2.75 (3H, s, 5-Me), 3.65 (3H, s, OMe), 7.28 (1H, s, metheneH), 8.92 br (2H, NH).

This dipyrromethene may be obtained in equally good yield by replacing the



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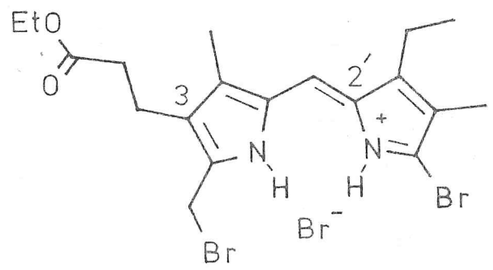
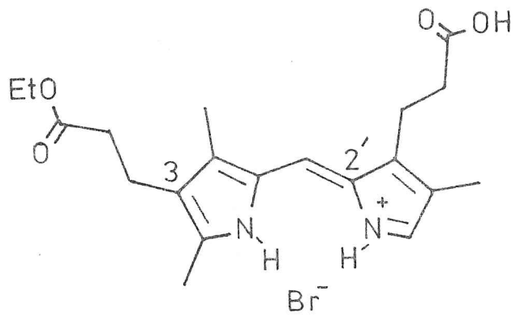


(40)

pyrrole ^{t}Bu ester (2b) for the pyrrole acid (3) or the pyrrole (6b) for the aldehyde (7) in the above procedure. However, the reaction between (2b) and (6b) themselves under similar conditions gave only poor yields.

Ethyl 5-[[5-Carboxy-3-ethyl-4-methyl-2H-pyrrol-2-ylidene]methyl]-2,4-dimethyl-1H-pyrrole-3-propanoate, monohydrobromide (39).- Replacing the methyl ester (3) by the ethyl ester (38) in the above procedure for (15) gave the dipyrromethene in 92% yield, m.p. 130-134° (Found: C, 54.41; H, 6.33; N, 6.30. $C_{20}H_{27}N_2O_4Br$ requires C, 54.67; H, 6.20; N, 6.38%), $\lambda_{max.}$ 408 and 479 nm; $\nu_{max.}$ 1 730, 1 715, and 1 630 cm^{-1}; δ 1.18 (3H, t, J 7 Hz, Et CH_3), 1.27 (3H, t, J 7 Hz, ester Me), 2.3 (3H, s, 4'-Me), 2.42 (3H, s, 3-Me), 2.6 (2H, m, Et CH_2), 2.7 (4H, m, CH_2CH_2COO), 2.79 (3H, s, 5-Me), 4.14 (2H, q, J 7 Hz, ester CH_2), 7.28 (1H, s, metheneH), 8.82 br (2H, NH).

Ethyl 2-(bromomethyl)-5-[[5-bromo-3-ethyl-4-methyl-2H-pyrrol-2-ylidene]methyl]-4-methyl-1H-pyrrole-3-propanoate, monohydrobromide (40).- A solution of the dipyrromethene (39) (5 g, 11.4 mM) in dry 1,2-dichloroethane (60 ml) was warmed to 80° (thermostatted oil-bath temperature) and bromine (30 g) in dry 1,2-dichloroethane (30 ml) added. After exactly 1 h at that temperature, the mixture was rapidly cooled and the solvent evaporated. The residue was stirred for 5 min with 1,2-dichloroethane (20 ml) and cyclohexene (20 ml) to destroy excess bromine and the volatile material evaporated under high vacuum. Crystallisation from 1,2-dichloroethane afforded the dibromo dipyrromethene (4.57 g, 72.5%), m.p.>300° (Found: C, 40.99; H, 4.83; N, 4.81. $C_{19}H_{25}N_2O_2Br_3$ requires C, 41.25; H, 4.56; N, 5.07%), $\lambda_{max.}$ 448 nm; $\nu_{max.}$ 1 725 and 1 620 cm^{-1}; δ 1.17 (3H, t, J 7 Hz, ester Me), 1.22 (3H, t, J 7 Hz, Et CH_3), 2.08 (3H, s, 4'-Me), 2.35 (3H, s, 3-Me), 2.6 (2H, m, $CH_2\underline{CH_2}COO$), 2.86 (2H, m, $\underline{CH_2}CH_2COO$), 2.8 (2H, q, J 7 Hz, Et CH_2), 4.14 (2H, q, J 7 Hz, ester CH_2), 4.96 (2H, s, CH_2Br), 7.18 (1H, s, methene H).



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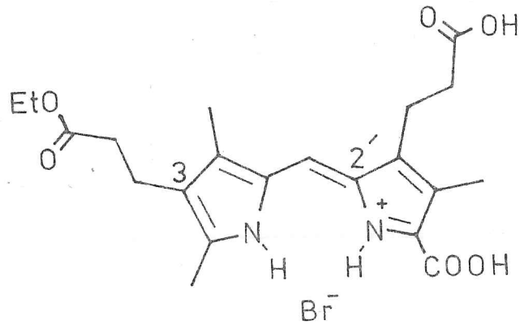
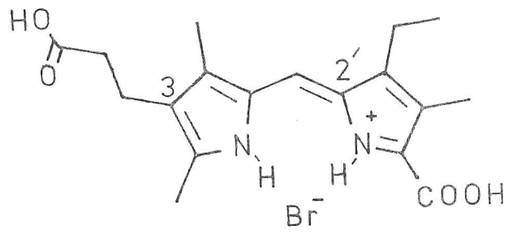


(52)

Ethyl 5-[[3-(2-Carboxyethyl)-4-methyl-2H-pyrrol-2-ylidene]methyl]-2,4-dimethyl-1H-pyrrole-3-propanoate, monohydrobromide (48).- Hydrogen bromide in acetic acid (5 ml of a 48% solution) was added to a stirred solution of the pyrrole acid (38) (2.59 g, 11 mM) and the pyrrole aldehyde (45) (1.96 g, 11 mM) in acetic acid (7 ml) at 20°. Stirring was continued for ½ h, the solvent evaporated, and the residue crystallised from chloroform / ether to give the α-free dipyrromethene (4 g, 84%), m.p. 162-164.5° (Found: C, 54.46; H, 6.17; N, 6.30. $C_{20}H_{27}N_2O_4Br$ requires C, 54.69; H, 6.20; N, 6.38%), $\lambda_{max.}$ 472 nm; $\nu_{max.}$ 1 740, 1 695, and 1 630 cm^{-1}; δ 1.26 (3H, t, J 7 Hz, Et CH_3), 2.1 (3H, s, 4'-Me), 2.35 (3H, s, 3-Me), 2.5 (4H, m, CH_2COO), 2.7 (2H, m, 4-CH_2), 2.74 (3H, s, 5-Me), 3.03 (2H, m, 3'-CH_2), 4.14 (2H, q, J 7 Hz, Et CH_2), 7.4 (1H, s, metheneH), 7.73 (1H, d, α-H).

Ethyl 5-[[5-Carboxy-3-(2-carboxyethyl)-4-methyl-2H-pyrrol-2-ylidene]methyl]-2,4-dimethyl-1H-pyrrole-3-propanoate, monohydrobromide (52).- The pyrrole acid (38) (3.11 g, 13 mM) and the aldehyde (51) (2.93 g, 13 mM) were treated as described for the formation of (48) above, and yielded the dipyrromethene (5.6 g, 89%), m.p. 129-132° (Found C, 51.93; H, 5.70; N, 5.70. $C_{21}H_{27}N_2O_6Br$ requires C, 52.18; H, 5.63; N, 5.80%), $\lambda_{max.}$ 400 and 484 nm; $\nu_{max.}$ 1 760, 1 720, 1 675, and 1 625 cm^{-1}; δ (CF_3COOD) 1.38 (3H, t, J 7 Hz, Et CH_3), 2.48 (3H, s, 3-Me), 2.53 (3H, s, 4'-Me), 2.78 (3H, s, 5-Me), 2.6 to 3.3 (8H, m, CH_2CH_2COO), 4.33 (2H, q, J 7 Hz, Et CH_2), 7.84 (1H, s, methene H).

5-[[5-Carboxy-3-ethyl-4-methyl-2H-pyrrol-2-ylidene]methyl]-2,4-dimethyl-1H-pyrrole-3-propanoic acid, monohydrobromide (60).- Hydrogen bromide in acetic acid (20 ml of a 48% solution) was added to a stirred solution of the pyrrole half ester (59) (8.01 g, 0.03 M) and the pyrrole aldehyde (7) (5.43 g, 0.03 M) in acetic acid (20 ml) at 20°. Stirring was continued for 20 min, and the product crystallised from the solution. Precipitation was completed



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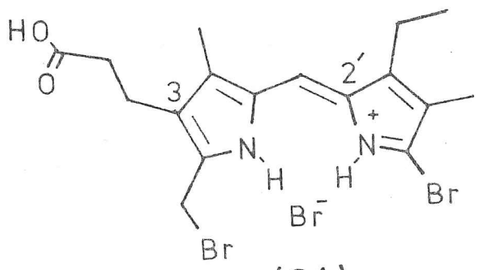

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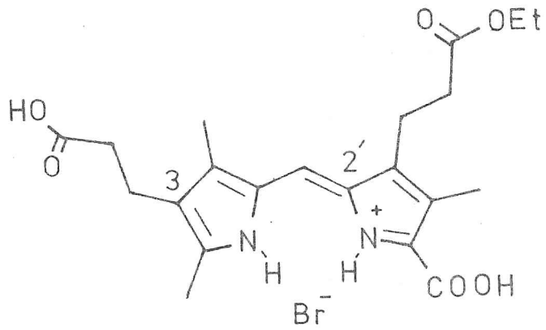
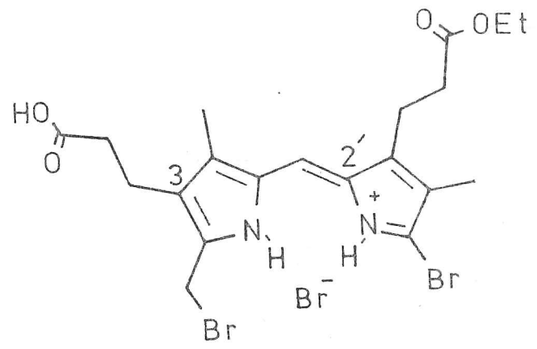


(62)

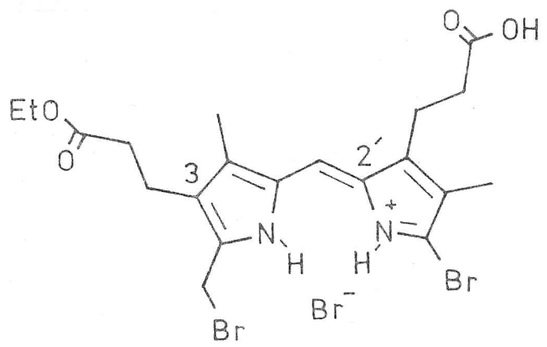
by the addition of ether (80 ml). The filtered product was dried under high vacuum and gave 11.08 g (90%), m.p. 166-168° (lit.,[127] 165-170°), $\lambda_{max.}$ 407 and 486 nm; $\nu_{max.}$ 1 710, 1 670, and 1 630 cm^{-1}; δ (CF_3COOD) 1.27 (3H, t, J 7 Hz, Et CH_3), 2.43 (3H, s, 4'-Me), 2.52 (3H, s, 3-Me), 2.88 (3H, s, 5-Me), 2.7 to 3.1 (4H, m, CH_2CH_2COO), 2.9 (2H, q, J 7 Hz, Et CH_2), 7.62 (1H, s, methene H).

2-(Bromomethyl)-5-[[5-bromo-3-ethyl-4-methyl-2H-pyrrol-2-ylidene]methyl]-4-methyl-1H-pyrrole-3-propanoic acid, monohydrobromide (61).- Treatment of the foregoing free acid (60) (5g, 12 mM) in essentially the same way as described above for (40), except that heating was continued for 3 h, gave the desired product (5.76 g, 90%), after evaporation of solvent. Due to its low solubility, it was not crystallised, but washed well with ether to remove contaminating material. N.m.r. revealed that it still contained a small (5%) quantity of monobrominated compound. The pure product was not characterised: this preparation was of sufficient purity for further reaction (see below). δ(CF_3COOD) 1.32 (3H, t, J 7 Hz, Et CH_3), 2.18 (3H, s, 4'-Me), 2.44 (3H, s, 3-Me), 2.9 (2H, m, Et CH_2), 2.7 to 3.1 (4H, m, CH_2CH_2COO), 4.78 (2H, s, CH_2Br), 7.5 (1H, s, methene H). Impurity had δ 2.66 and 7.39 (s) characteristically.

5-[[5-Carboxy-3-(2-ethoxycarbonylethyl)-4-methyl-2H-pyrrol-2-ylidene]methyl]-2,4-dimethyl-1H-pyrrole-3-propanoic acid, monohydrobromide (62).- The pyrrole half ester (59) (6.74 g, 25 mM) and the aldehyde (42) (6.38 g, 25 mM) were treated as described for the preparation of (60) to give the dipyrromethene (11.46 g, 94%), m.p. 139-143°, $\lambda_{max.}$ 402, 486 nm; $\nu_{max.}$ 1 710, 1 680, and 1 620 cm^{-1}; δ (CF_3COOD) 1.33 (3H, t, J 7 Hz, Et CH_3), 2.46 (3H, s, 3-Me), 2.56 (3H, s, 4 -Me), 2.8 (3H, s, 5-Me), 2.6 to 3.3 (8H, m, CH_2CH_2COO), 4.28 (2H, q, J 7 Hz, Et CH_2), 7.72 (1H, s, methene H).



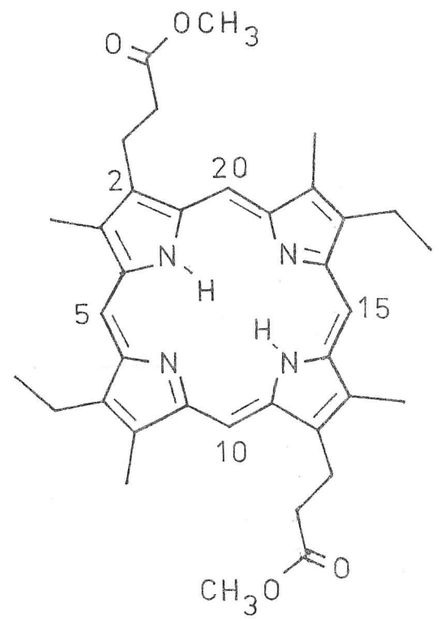
(66)



(70)

2-(Bromomethyl)-5-[[5-bromo-3-(2-ethoxycarbonylethyl)-4-methyl-2H-pyrrol-2-ylidene]methyl]-4-methyl-1H-pyrrole-3-propanoic acid, monohydrobromide (66).- The foregoing dipyrromethene (62) (6 g, 12.4 mM) was treated as described for (40) above and gave the dibromo compound (5.91 g, 79.7%). As with (61), the pure compound was not characterised: this preparation sufficed for the further reaction to form porphyrin (see below). δ (CF_3COOD) 1.38 (3H, t, Et CH_3), 2.24 (3H, s, 3-Me), 2.51 (3H, s, 4'-Me), 2.8 to 3.4 (8H, m, CH_2CH_2COO), 4.34 (2H, q, Et CH_2), 4.84 (2H, s, CH_2Br), 7.62 (1H, s, methene H).

Ethyl 2-(Bromomethyl)-5-[[5-bromo-3-(2-carboxyethyl)-4-methyl-2H-pyrrol-2-ylidene]methyl]-4-methyl-1H-pyrrole-3-propanoate, monohydrobromide (70).- The dipyrromethene (52) (5.8 g, 12 mM) was treated as described for (40) above and gave the dibromo compound (3.7 g, 51.6%). As with (61), the pure compound was not characterised: this preparation sufficed for the further reaction to form porphyrin (see below). δ(CF_3COOD) 1.41 (3H, t, J 7 Hz, Et CH_3), 2.22 (3H, s, 3-Me), 2.47 (3H, s, 4'-Me), 2.7 to 3.4 (8H, m, CH_2CH_2COO), 4.37 (2H, q, J 7 Hz, Et CH_2), 4.78 (2H, s, CH_2Br), 7.7 (1H, s, methene H).



(16)

d) Porphyrins

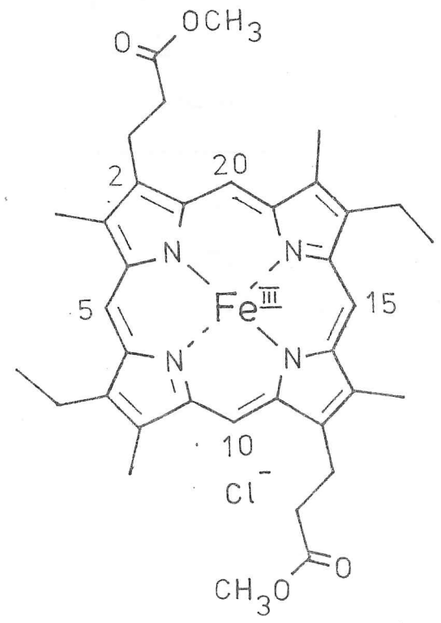
Mesoporphyrin II, dimethyl ester (16).- Bromine (8.55 g, 54mM) in formic acid (25 ml) was added at 20° to the α-free dipyrromethene (14) (10.18 g, 27 mM) in formic acid (25 ml) over 15 min, with stirring. Acetic anhydride (5 ml) was then added and the mixture heated at reflux for 3 h. On cooling and evaporating solvent, a dark gum was produced, to which methanol (50 ml) and conc. sulphuric acid (0.5 ml) were added and the solution allowed to stir for 18 h. The porphyrin was then extracted into chloroform and the organic layer washed with water. Evaporation of the solvent afforded the crude product, which was crystallised from hot chloroform (40 ml) by addition of methanol (40 ml). The solid material thus produced was recrystallised from chloroform / methanol. The mother liquors were combined and the residue after evaporation chromatographed on neutral alumina (150 g, grade 3), eluting with dichloromethane, to give more product after crystallisation. The overall yield was 3.07 g (38.6%), m.p. 232-233° (lit., [52] 233°)(Found: C, 72.84; H, 7.32; N, 9.36. $C_{36}H_{42}N_4O_4$ requires C, 72.70; H, 7.12; N, 9.42%), $\lambda_{max.}$ 299 (log ε 4.07), 407 (5.2), 500 (3.87), 534 (3.75), 568 (3.65), 599 (3.08), and 624 nm (3.4); $\nu_{max.}$ 1735 cm^{-1}; m/e 594 (M+, 100%), 579 (5), 563 (5), 535 (12), and 521 (20), m* 457 (594 → 521); δ ($CDCl_3$) -3.8 (2H, s, NH), 1.85 (6H, t, J 7.5 Hz, Et CH_3), 3.23 (4H, t, J 7.1 Hz, CH_2COO), 3.57 and 3.61 (each 6H, s, ring Me), 3.68 (6H, s, ester Me), 4.06 (4H, q, J 7.5 Hz, Et CH_2), 4.34 (4H, t, J 7.1 Hz, $C\underline{H}_2CH_2COO$), 10.00 (4H, s, mesoH). δ (Pyridine-d_5) -3.13 (2H, s, NH), 1.85 (6H, t, J 7.5 Hz, Et CH_3), 3.44 (4H, t, J 7.1 Hz, CH_2COO), 3.59 and 3.61 (18H, ring Me, ester Me), 4.09 (4H, q, J 7.5 Hz, Et CH_2), 4.54 (4H, t, J 7.1 Hz, $C\underline{H}_2CH_2COO$), 10.35 (2H, s, 5, 15-H), 10.45 (2H, s, 10, 20-H).

Using the 5-carboxy dipyrromethene (15) (16.8 g, 0.04 M) in the above procedure gave 3.13 g (26.6%) of (16). Alternatively, the bis-acid dipyrromethene (60) (18.2 g, 0.044 M) gave 5.03 g (38.2%) when likewise employed.

It was found unnecessary to isolate the dipyrromethenes: the following procedure produces (16) directly from pyrroles.

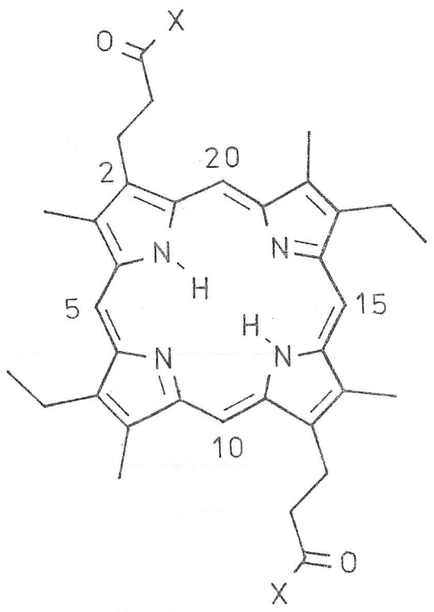
To a solution of the pyrrole (3) (5.625 g, 0.025 M) and the pyrrole aldehyde (7) (4.525 g, 0.025 M) in formic acid (20 ml) was added hydrogen bromide in acetic acid (12.5 ml of a 48% solution). After stirring for $\frac{1}{2}$ h, bromine (8 g, 0.05 M) in formic acid (20 ml) was added and the mixture heated at reflux for 3 h. Work-up was essentially as described above for (16) and yielded 2.2 g (29.6%).
The benzyl ester (6a) of the pyrrole aldehyde may replace it in this sequence, but the yield is then lower (16%).

Mesoporphyrin II, dimethyl ester, Iron (III) complex, chloride (16a).-



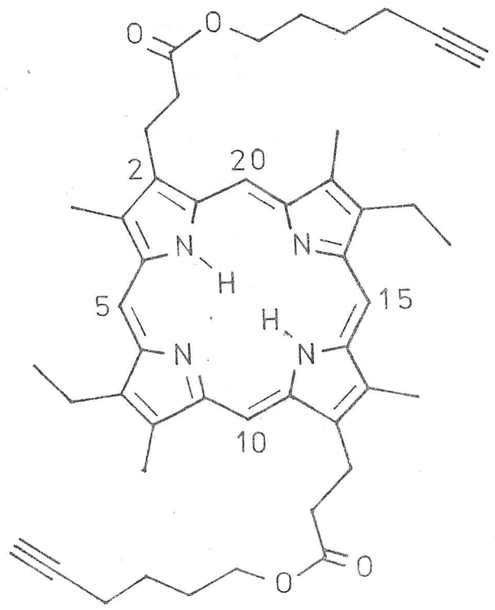
(16a)

The above ester (16) (1 g, 1.68 mM) in pyridine (2.5 ml) and acetic acid (30 ml) was treated with ferrous sulphate (0.5 g) at 80°. After stirring for 15 min, a mixture of saturated brine (10 ml) and water (10 ml) was added, and the whole allowed to cool. The precipitated product was washed with water and dried under high vacuum to give 1.09 g (95%), m.p. >300° (Found: C, 63.00; H, 5.93; N, 8.28. $C_{36}H_{40}N_4O_4FeCl$ requires C, 63.20; H, 5.89; N, 8.28%), $\lambda_{max.}$ 376, 506, 537, and 638 nm; $\nu_{max.}$ 1 725 cm^{-1}; δ ($(CD_3)_2SO$ saturated with KCN) -0.50 (6H, t, J 6.5 Hz, Et CH_3), -0.15 (4H, t, J 5 Hz, CH_2COO), 0.23 and 0.82 (each 2H, s, mesoH), 3.15 (6H, s, ester Me), 4.90 (4H, t, J 5 Hz, CH_2CH_2COO), 6.15 (4H, q, J 6.5 Hz, Et CH_2), 11.95 and 14.20 (each 6H, s, ring Me).
The corresponding Fe (II) material was prepared in pyridine with anhydrous hydrazine and had δ 1.86 (6H, t, J 7.5 Hz, Et CH_3), 3.42 (4H, t, J 7.5 Hz, CH_2COO), 3.50 (12H, s, ring Me), 3.53 (6H, s, ester Me), 3.97 (4H, q, J 7.5 Hz, Et CH_2), 4.45 (4H, t, J 7.5 Hz, CH_2CH_2COO), 9.95 (2H, s, 5, 15-H), 10.03 (2H, s, 10,20-H).



(19) X = OH

(20) X = Cl

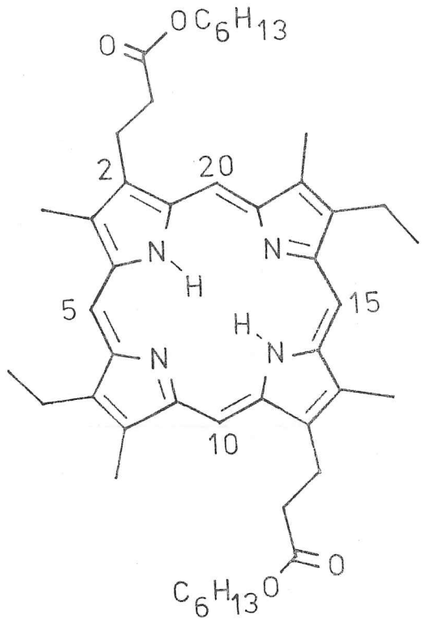


(21)

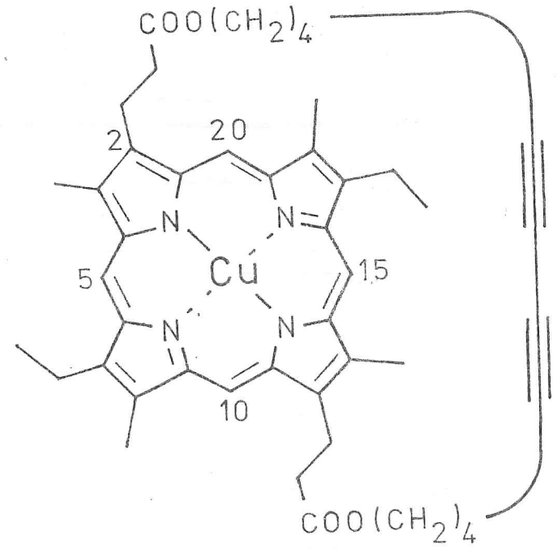
Mesoporphyrin II (19).- The above ester (16) (5 g, 8.42 mM) was stirred in 6N hydrochloric acid (150 ml) at 20° for 18 h, protected from the light. The acid was removed by evaporation and the residue crystallised from pyridine / methanol to give the porphyrin free acid [52] (4.63 g, 97%), m.p. 310° (Found: C, 72.21; H, 7.00; N, 9.63. $C_{34}H_{38}N_4O_4$ requires C, 72.06; H, 6.76; N, 9.89%), $\lambda_{max.}$ 400, 500, 534, 571, and 626 nm; $\nu_{max.}$ 1 710 cm^{-1}; δ -3.08 (2H, s, NH), 1.84 (6H, t, J 7.5 Hz, Et CH_3), 3.50 (4H, t, J 7.3 Hz, 3.56 (6H, s, 8, 18-Me), 3.68 (6H, s, 3, 13-Me), 4.07 (4H, q, J 7.5 Hz, Et CH_2), 4.69 (4H, t, J 7.3 Hz, CH_2CH_2COO), 10.35 (2H, s, 5, 15-H), 10.58 (2H, s, 10, 20-H).

Mesoporphyrin II, bis acid chloride (20).- The acid (19) (1 g, 1.77 mM) was dissolved in thionyl chloride (5 ml) containing dimethylformamide (2 drops) and stirred at 20° for 2 h while protected from the light. The excess reagent was evaporated and the crude product, a red gum, was not characterised but used immediately in the various preparations described below.

Mesoporphyrin II, dihex-5-yn-1-yl ester (21).- The crude bis acid chloride (20), prepared as described above from (19) (4.63 g, 8.18 mM) was dissolved in dichloromethane (10 ml) and treated with hex-5-yn-1-ol (18) (5 ml) at 20° by stirring for 1½ h. Pyridine (10 ml) in dichloromethane (20 ml) was then added, with external ice-cooling, and the mixture kept at 0° for 18 h. The product was extracted into dichloromethane and the organic layer washed with 1N sulphuric acid and water. After evaporation and crystallisation from chloroform / ether, 5.19 g (87.4%) of the diester was obtained, m.p. 172-173.5° (Found: C, 76.04; H, 7.55; N, 7.67. $C_{46}H_{54}N_4O_4$ requires C, 76.00; H, 7.50; N, 7.70%), $\lambda_{max.}$ 402, 502, 538, 572, and 626 nm; $\nu_{max.}$ 3 300, 2 100, and 1 720 cm^{-1}; m/e 726 (M+), 660, 646, and 587; δ -3.14 (2H, s, NH), 1.3 to 1.6 (8H, m, alkylCH_2), 1.84 (6H, t, J 7.5 Hz, Et CH_3),



(21a)

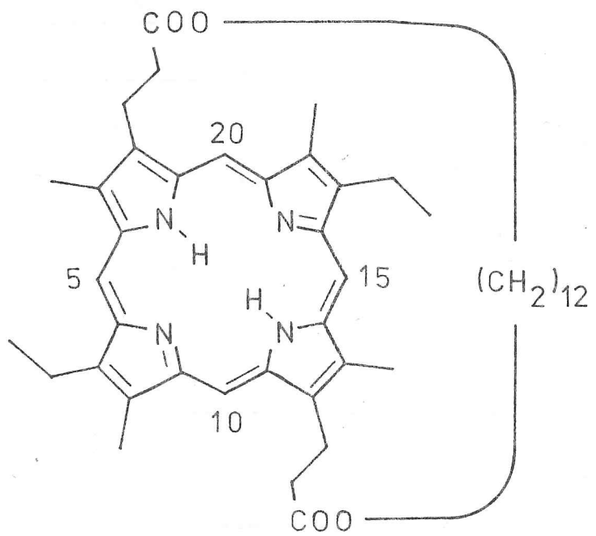


(22)

1.88 (4H, m, $CH_2C\equiv C$), 2.51 (2H, t, J 2.6 Hz, $C\equiv CH$), 3.46 (4H, t, J 7.5 Hz, CH_2COO), 3.59 (6H, s, 8,18-Me), 3.62 (6H, s, 3,13-Me), 4.06 (4H, q, J 7.5 Hz, Et CH_2), 4.12 (4H, t, J 6.5 Hz, $COOCH_2$), 4.56 (4H, t, J 7.5 Hz, $\underline{CH_2}CH_2COO$), 10.35 (2H, s, 5,15-H), 10.45 (2H, s, 10,20-H).

Mesoporphyrin II, dihexyl ester (21a).- The foregoing acetylenic ester (21) (0.38 g, 0.53 mM) was stirred at 20° in tetrahydrofuran (50 ml) with 10% palladised charcoal (0.1 g) and hydrogen until uptake had ceased (2 h). After filtration and evaporation of the solvent, the residue was crystallised from chloroform / methanol and gave 258 mg (67%) of the diester, m.p. 156-159° (Found: C, 74.93; H, 8.48; N, 7.59. $C_{46}H_{62}N_4O_4$ requires C, 75.17; H, 8.50; N, 7.62%), $\lambda_{max.}$ 1 730 cm^{-1}; δ -3.14 (2H, s, NH), 0.52 (6H, m, alkyl CH_3), 0.85 br (12H, m, alkylCH_2), 1.33 (4H, m, $COOCH_2\underline{CH_2}$), 1.84 (6H, t, J 7.5 Hz, Et CH_3), 3.48 (4H, t, J 7.5 Hz, CH_2COO), 3.59 (6H, s, 8,18-Me), 3.63 (6H, s, 3,13-Me), 4.06 (4H, t, J 7.5 Hz, Et CH_2), 4.09 (4H, t, J 6.5 Hz, $COOCH_2$), 4.57 (4H, t, J 7.5 Hz, $\underline{CH_2}CH_2COO$), 10.34 (2H, s, 5,15-H), 10.46 (2H, s, 10,20-H).

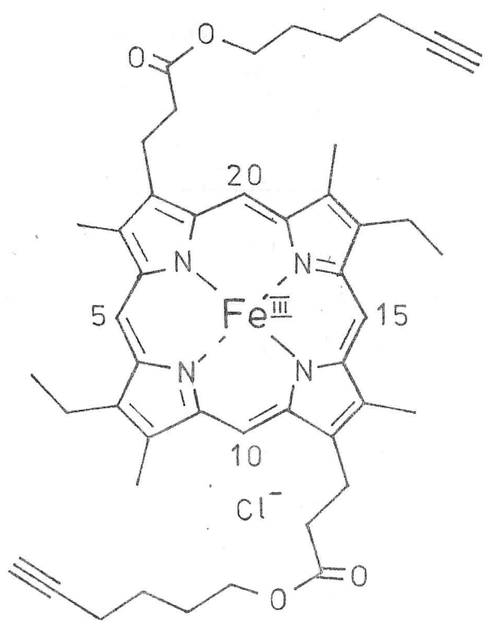
Mesoporphyrin II, dodeca-5,7-diyn-1,12-diol, cyclic diester, copper complex (22).- The foregoing acetylenic ester (21) (0.5 g, 0.69 mM) was placed in a Soxhlet thimble above a mixture of anhydrous copper (II) acetate (0.81 g, 4.46 mM), pyridine (110 ml), and ether (50 ml). The reaction flask was heated at reflux so that the descending condensate (mainly ether) slowly extracted the porphyrin into the reagent solution. After 4 days, 30 mg remained in the thimble. The reaction mixture was cooled, evaporated to low bulk, and the porphyrin extracted into chloroform. The organic layer was washed with water, 1N hydrochloric acid and brine. Evaporation afforded a gum which was chromatographed on neutral alumina (30 g, grade 3), eluting with dichloromethane. The blood-red product was crystallised from chloroform / ether



(23)

and yielded 0.31 g (60%), m.p. 174-177° (Found: C, 70.04; H, 6.54; N, 6.98. $C_{46}H_{50}N_4O_4Cu$ requires C, 70.25; H, 6.41; N, 7.12%), $\lambda_{max.}$ 286 (log ∈ 3.71), 327 (4.10), 402 (5.46), 531 (3.97), and 569 nm (4.26); $\nu_{max.}$ 1 725 cm^{-1}; m/e 785.3146 (requires 785.3126), 785 (M+, 100%), 493 (10), and 393 (10).

Mesoporphyrin II, dodeca-1,12-diol, cyclic diester (23).- The above copper chelate (22) (0.2 g, 0.26 mM) was stirred in dry tetrahydrofuran with 10% palladised charcoal (40 mg) and hydrogen for 1 h at 20° (uptake was then complete). The filtered solution was evaporated and the residue treated with trifluoroacetic acid (2 ml). After 15 min, dichloromethane (25 ml) and water (75 ml) were added and the acid neutralised by addition of sodium carbonate. The porphyrin was extracted into the dichloromethane and the organic layer washed with more water before evaporation. The residue was again treated with trifluoroacetic acid: the whole procedure being repeated seven times until the visible spectrum confirmed the absence of copper-containing material. The residue was chromatographed on neutral alumina (30 g, grade 3), and the porphyrin fraction, eluted with dichloromethane, recrystallised twice from chloroform / methanol to yield the cyclic diester (80 mg, 42%), m.p. 221-223° (Found: C, 75.16; H, 8.51; N, 7.43. $C_{46}H_{60}N_4O_4$ requires C, 75.37; H, 8.25; N, 7.64%), $\lambda_{max.}$ 399, 500, 535, 571, and 623 nm; $\nu_{max.}$ 1 725 cm^{-1}; m/e 732 (M+); δ ($CDCl_3$) -3.8 (2H, s, NH), -0.71, -0.16, and 0.75 (20H, m, bridge H), 1.89 (6H, t, J 7.5 Hz, Et CH_3), 3.3 (4H, m, CH_2COO), 3.63 and 3.66 (each 6H, s, ring Me), 3.69 (4H, m, $COOCH_2$), 4.1 (6H, m, Et CH_2 and CH_2CH_2COO), 4.7 (2H, m, CH_2CH_2COO), 10.06 and 10.10 (each 2H, s, mesoH). δ (d_5-pyridine) -3.19 (2H, s, NH), -0.95, 0.10, and 0.82 (20H, m, bridge H), 1.86 (6H, t, J 7.5 Hz, Et CH_3), 3.52 (4H, m, CH_2COO), 3.65 (12H, m, ring Me), 3.90 (4H, t, J 6 Hz, $COOCH_2$), 4.09 (4H, q, J 7.5 Hz, Et CH_2), 4.2 and 4.6 (each 2H, m, CH_2CH_2COO), 10.31 (2H, s, 5,15-H), 10.47 (2H, s, 10,20-H).



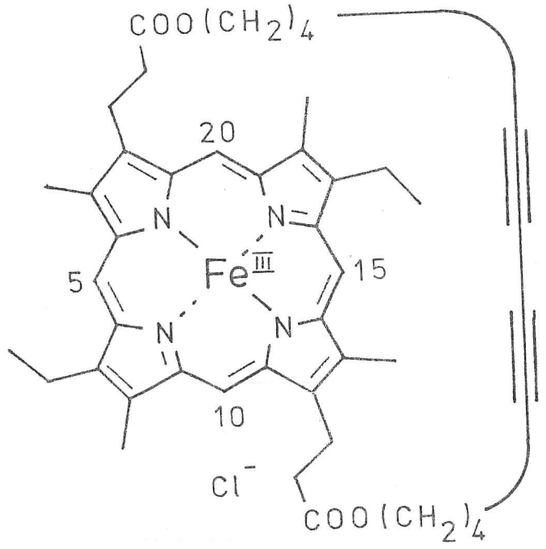
(24)

This compound was also prepared from the corresponding iron adduct (see below). The following procedure produced it by an independent route.

Mesoporphyrin II bis acid chloride (20) (from 0.3 g, 0.53 mM of (19)) was dissolved in dry dichloromethane (200 ml). This solution was slowly added, with stirring, to dry dichloromethane (150 ml) in a 1-l three-necked flask, while dodecane-1,12-diol (111 mg, 0.55 mM) in dry dichloromethane (200 ml) containing pyridine (1 ml) was simultaneously added. The rates of addition of the two solutions were maintained equal, and this was complete in 4 h. After a further 2 h, the solution was evaporated to low bulk and washed with water. The organic layer was evaporated and the residue chromatographed on Silica (60 g Kieselgel), eluting with chloroform. The first porphyrin eluted was product (220 mg, 56.7%) and was crystallised from chloroform / ether to give 173 mg (44.6%) of the cyclised diester, identical in all respects to that produced above.

Dihex-5-yn-1-yl mesoporphyrin II, Iron (III) complex, chloride (24).- The porphyrin diester (21) (5.2 g, 7.16 mM) in pyridine (10 ml) and acetic acid (100 ml) was treated with ferrous sulphate (2.5 g) at 80° for 15 min. A mixture of water (40 ml) and saturated aqueous sodium chloride (40 ml) was added and the whole set aside to cool. The precipitated iron complex was isolated by filtration, washed with water, and dried under high vacuum. It had m.p.>300° (Found: C, 66.80; H, 6.19; N, 7.07. $C_{46}H_{52}N_4O_4ClFe.\frac{1}{2}H_2O$ requires C, 66.94; H, 6.47; N, 6.79%), $\lambda_{max.}$ 380, 510, 540, and 643 nm.

Mesoporphyrin II, dodeca-5,7-diyn-1,12-diol cyclic diester, Iron (III) complex, chloride (25).- The above iron complex (24) (from 5.2 g of (21)) was treated essentially as described above for the formation of (22) in pyridine (500 ml) and ether (200 ml). 150 mg of material remained unreacted after 4 days. After chromatography and crystallisation (as for (24)) the



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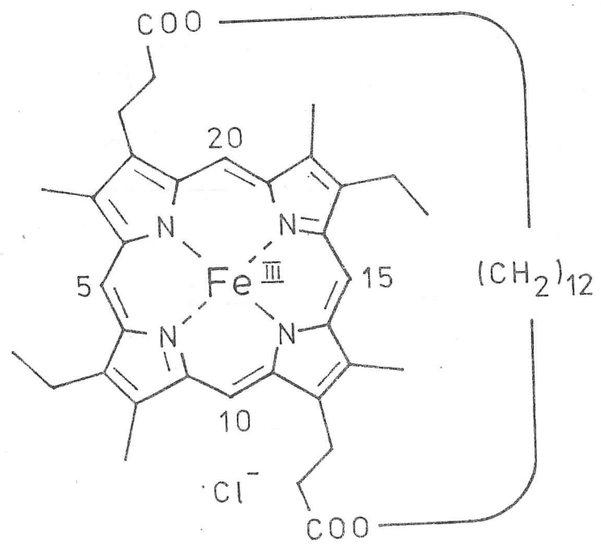


(26)

cyclised iron complex was obtained (2.427 g, 41.8% from (21)), m.p.>300° (Found: C, 66.62; H, 6.12; N, 6.80; Cl, 4.04. $C_{46}H_{50}N_4O_4ClFe.H_2O$ requires C, 66.38; H, 6.30; N, 6.73; Cl 4.26%), $\lambda_{max.}$ (acetone) 374 (log ϵ 4.56), 505 (3.54), 535 (3.55), and 634 nm (3.45); $\nu_{max.}$ 1 732 cm^{-1}.

The corresponding Fe (II) material was prepared in pyridine with anhydrous hydrazine and had δ 0.6 to 1.8 (12H, m, bridgeH), 1.9 (6H, t, J 7.5 Hz, Et CH_3), 3.4 and 3.6 (each 6H, s, ring Me), 3.5 (8H, m, CH_2COO and $COOCH_2$), 4.0 (4H, q, J 7.5 Hz, Et CH_2), 4.5 (4H, m, CH_2CH_2COO), 9.90 (4H, s, mesoH).

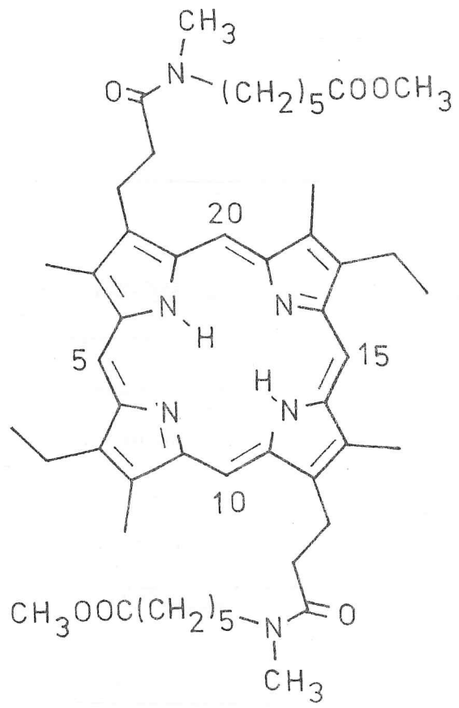
Mesoporphyrin II, dodeca-1,12-diol cyclic diester, Iron (III) complex, chloride (26).- The above iron complex (25) (0.75 g, 0.9 mM) was stirred in dry tetrahydrofuran (150 ml) with 10% palladised charcoal (100 mg) and hydrogen for 2 h at 20° (uptake was then complete). The catalyst was removed by filtration and the solvent evaporated. The resultant iron complex (0.75 g, 100%) had m.p. > 300° (Found: C, 67.15; H, 6.98; N, 6.56. $C_{46}H_{58}N_4O_4FeCl$ requires C, 67.18; H, 7.11; N, 6.81%), $\lambda_{max.}$ 374, 504, 535, and 634 nm; $\nu_{max.}$ 1 730 cm^{-1}.

The corresponding Fe (II) material was prepared in pyridine with anhydrous hydrazine and had δ 0.2 to 1.5 (20H, m, bridgeH), 1.89 (6H, t, J 7.5 Hz, Et CH_3), 3.5 (8H, m, CH_2COO and $COOCH_2$), 3.52 (12H, s, ring Me), 3.98 (4H, q, J 7.5 Hz, Et CH_2), 4.5 (4H, m, CH_2CH_2COO), 9.88 and 9.98 (each 2H, s, mesoH).

The metal-free material (23) was prepared from this complex by demetallation as follows:

The above iron complex (26) (0.2 g, 0.24 mM) was dissolved in pyridine (1 ml) and acetic acid (10 ml). Hydrogen bromide in acetic acid (4 ml of a 48% solution) was added, followed by ferrous sulphate (2 g). The mixture was shaken in a stoppered flask for 3 min, and poured into a mixture of water (100 ml) and chloroform (100 ml). The organic layer was separated and washed with water. Evaporation then afforded partially demetallated material (judged by the visible spectrum). The whole procedure was then repeated to complete

(29)



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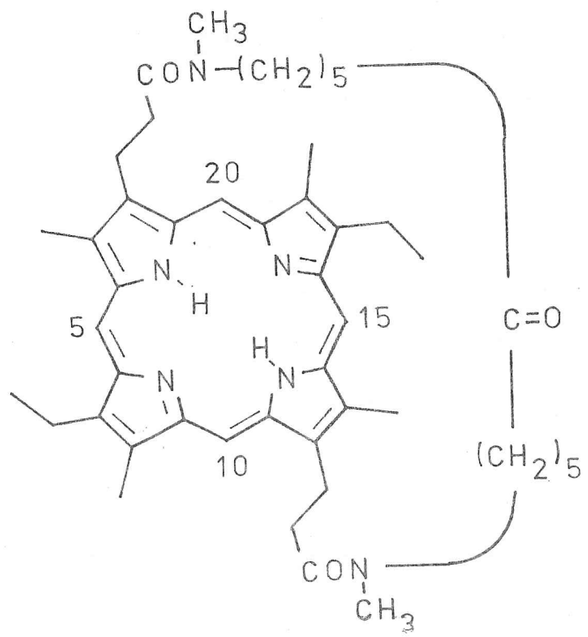
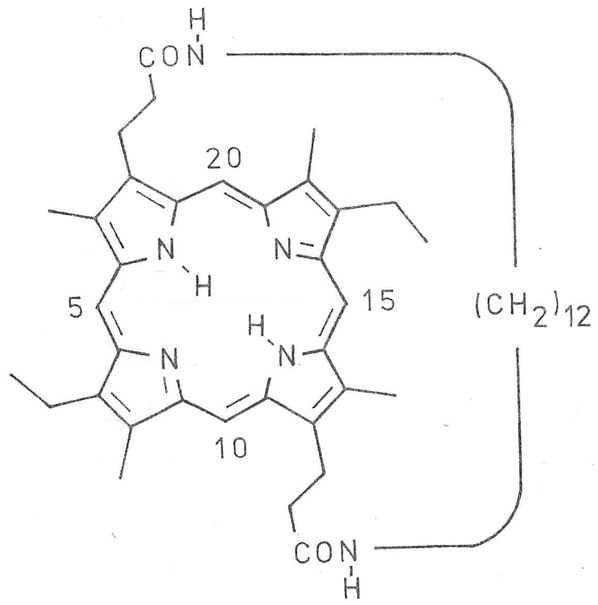


metal removal. The resultant porphyrin was purified by chromatography and crystallisation as for (23) (above) and gave material identical to that previously obtained by the other methods (140 mg, 80%).

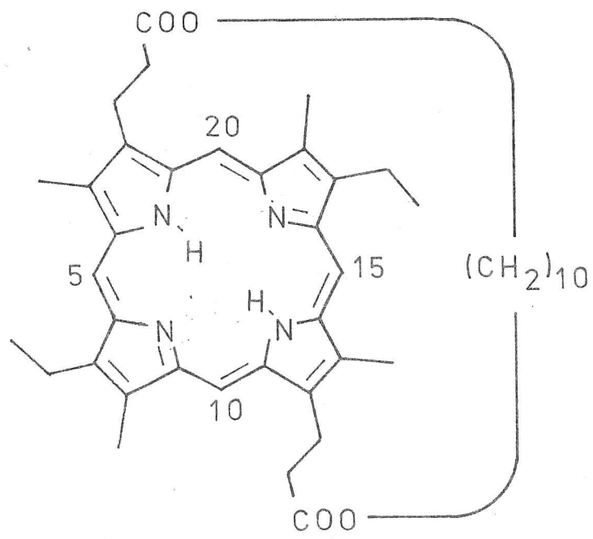
Mesoporphyrin II, bis amide with methyl 6-methylamino-hexanoate (29).- The amine hydrochloride (28) (1.13 g, 5.8 mM) was added to mesoporphyrin II bis acid chloride (20) (from 0.82g, 1.45 mM of (19)) in dichloromethane (10 ml). Pyridine (3 ml) was slowly added and the mixture stirred for 5 h. The product was extracted into chloroform and the organic layer washed with water. Evaporation gave a residue which was crystallised from methanol to yield the amide (775 mg, 63%), m.p. 118-120°, $\lambda_{max.}$ 405, 501, 535, 570, and 624 nm; $\nu_{max.}$ 1 720 and 1 630 cm^{-1}; m/e 848 (M+); δ -3.05 (2H, s, NH), 1.2 (12H, m, alkyl CH_2), 1.85 (6H, t, J 7.5 Hz, Et CH_3), 2.1 (4H, m, CH_2COO), 2.69 and 2.93 (each 3H, s, N-Me), 2.95 and 3.4 (each 2H, m, CH_2NCO), 3.38 and 3.55 (each 3H, ester Me), 3.42 (4H, m, CH_2CON), 3.61 and 3.66 (12H, ring Me), 4.06 (4H, q, J 7.5 Hz, Et CH_2), 4.69 (4H, t, J 7.1 Hz, CH_2CH_2CON), 10.38 (2H, s, 5,15-H), 10.58 (2H, s, 10,20-H).

Mesoporphyrin II, N,N-dimethyl 6-oxo-undeca-1,11-diamine, cyclic bis amide (30).- The foregoing porphyrin (29) (0.5 g, 0.59 mM) was cyclised in a high dilution apparatus with potassium tert-butoxide (from 0.7 g potassium) in toluene at reflux, by slow addition to a pre-mixing bulb (100 ml) from whence it was washed by the refluxing solvent into the reaction flask (which contained 300 ml toluene). Addition took 18 h. On completion, acetic acid (10 ml) was carefully added to the cooled solution, the solvent evaporated and the residue washed in dichloromethane with water. Evaporation and chromatography on Fluka neutral alumina (80 g, grade 3), eluting with 3% methanol in chloroform gave the cyclised product (125 mg, 28%), m.p. 133-135° (Found: m/e 758.4899. $C_{47}H_{62}N_6O_3$ requires 758.4884), $\lambda_{max.}$ 405, 502, 534, 570, and 624 nm;

(31)



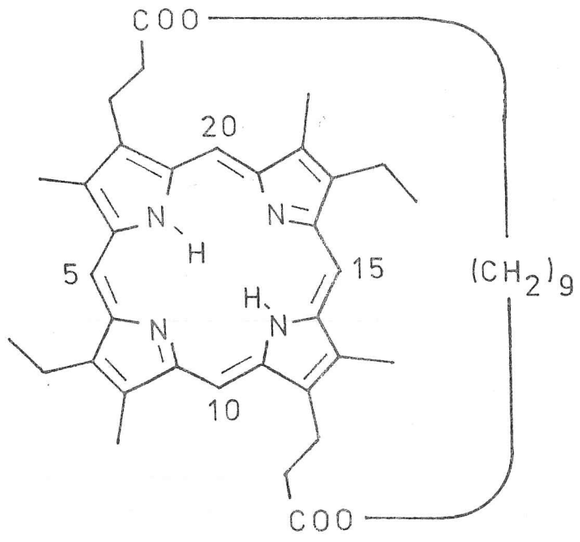
(32)



$\nu_{max.}$ 1 710 and 1 630 cm^{-1}; m/e 758 (M+); δ -3.16 (2H, s, NH), -1.5, -0.3, and 0.3 (16H, m, bridge H), 1.81 (6H, m, Et CH_3), 2.37 and 2.77 (6H, N-Me), 2.8 and 3.4 (4H, m, $CONCH_2$), 3.4 (4H, m, CH_2CON), 3.7 (12H, ring Me), 4.12 (4H, m, Et CH_2), 4.3 and 4.9 (each 2H, m, CH_2CH_2CON), 10.34, 10.64, and 10.77 (4H, meso H).

Mesoporphyrin II, dodeca-1,12-diamine, cyclic diamide (31).- When mesoporphyrin II bis acid chloride (20) (from 0.505 g, 0.89 mM of (19)) was reacted at high dilution with dodeca-1,12-diamine (0.357 g, 1.78 mM), as described above for the formation of (23), the crude product (234 mg, 36%) was obtained after chromatography on Fluka neutral alumina (50 g, grade 3). Crystallisation from chloroform / light petroleum (b.p. 60-80°) afforded the cyclised amide (172 mg, 26.4%), m.p.>300° (Found: C, 75.29; H, 8.38; N, 11.75. $C_{46}H_{62}N_6O_2$ requires C, 75.58; H, 8.55; N, 11.50%, m/e 730.4949, requires 730.4933), $\lambda_{max.}$ 402, 503, 537, 572, and 624 nm; $\nu_{max.}$ 1 635 and 1 540 cm^{-1}; m/e 730 (M+); δ -3.14 (2H, s, NH), -0.85, -0.05, and 0.84 (20H, m, bridge H), 1.90 (6H, t, J 7.5 Hz, Et CH_3), 3.28 (4H, m, CH_2CON), 3.56 (4H, m, $CONCH_2$), 3.62 (6H, s, 8,18-Me), 3.67 (6H, s, 3,13-Me), 4.12 (4H, q, J 7.5 Hz, Et CH_2), 4.2 and 4.9 (each 2H, m, CH_2CH_2CON), 8.25 (2H, t, J 5.5 Hz, CONH), 10.32 (2H, s, 5,15-H), 10.61 (2H, s, 10,20-H).

Mesoporphyrin II, decane-1,10-diol, cyclic diester (32).- Mesoporphyrin II (19) (150 mg, 0.27 mM) was treated as described above for the preparation of the bridged porphyrin (23), substituting decane-1,10-diol (55 mg, 0.32 mM) for the C_{12} diol, and gave 65 mg (34.8%) of the bridged adduct, m.p. 228-230° (from dichloromethane / ethanol) (Found: C, 74.90; H, 8.13; N, 7.69. $C_{44}H_{56}N_4O_4$ requires C, 74.96; H, 8.01; N, 7.95%), $\lambda_{max.}$ 400, 500, 534, 571, and 623 nm; $\nu_{max.}$ 3 300 and 1 725 cm^{-1}; m/e 704 (M+); δ -3.2 (2H, s, NH), -2.3, -1.7, -0.80, and 0.30 (16H, m, bridge H), 1.85 (6H, t, J 7.5 Hz, Et CH_3), 3.5 (4H, m, CH_2COO), 3.61 (6H, s, 8,18-Me), 3.68 (6H, s, 3,13-Me), 3.83 (4H, m, $COOCH_2$),



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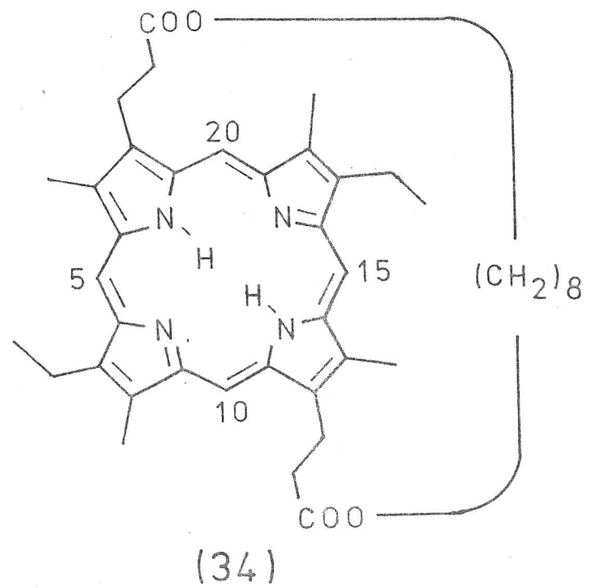
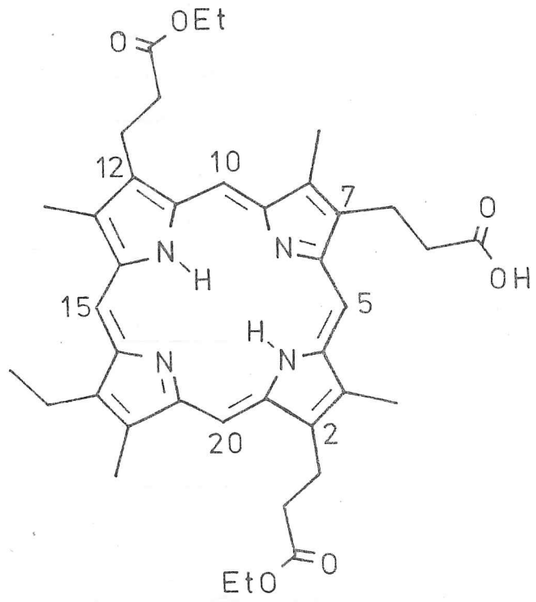


(34)

4.09 (4H, q, J 7.5 Hz, Et CH_2), 4.1 and 4.7 (each 2H, m, CH_2CH_2COO), 10.31 (2H, s, 5,15-H), 10.48 (2H, s, 10,20-H).

Mesoporphyrin II, nonane-1,9-diol, cyclic diester (33).- Mesoporphyrin II (19) (200 mg, 0.35 mM) was treated as described above for the preparation of the bridged porphyrin (23), substituting nonane-1,9-diol (85 mg, 0.53 mM) for the C_{12} diol and gave 100 mg (41%) of the bridged adduct, m.p. 263-266° (from dichloromethane / ethanol), $\lambda_{max.}$ 400, 500, 534, 570, and 623 nm; $\nu_{max.}$ 3 300 and 1 725 cm^{-1}; m/e 690 (M+); δ -3.2 (2H, s, NH), -3.2, -1.5, 0.15 (14H, m, bridge H), 1.84 (6H, t, J 7.5 Hz, Et CH_3), 3.4 (4H, m, CH_2COO), 3.59 (6H, s, 8,18-Me), 3.63 (4H, m, $COOCH_2$), 3.68 (6H, s, 3,13-Me), 4.08 (4H, q, J 7.5 Hz, Et CH_2), 4.1 and 4.7 (each 2H, m, CH_2CH_2COO), 10.32 (2H, s, 5, 15-H), 10.41 (2H, s, 10,20-H).

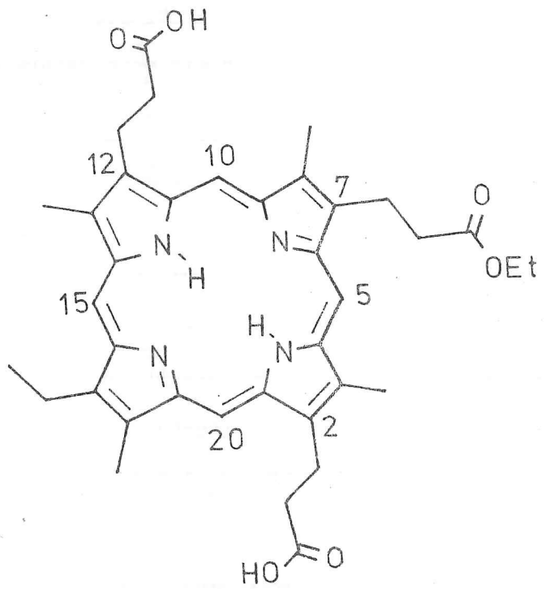
Mesoporphyrin II, octane-1,8-diol, cyclic diester (34).- Mesoporphyrin II (19) (300 mg, 0.53 mM) was treated as described above for the preparation of the bridged porphyrin (23), substituting octane-1,8-diol (116 mg, 0.8 mM) for the C_{12} diol, and gave 135 mg (37.7%) of the bridged adduct, m.p. 280-283° (Found: C, 74.39; H, 7.68; N, 8.24. $C_{42}H_{52}N_4O_4$ requires C, 74.52; H, 7.74; N, 8.28%), $\lambda_{max.}$ 400, 500, 534, 570, and 624 nm; $\nu_{max.}$ 3 300 and 1 725 cm^{-1}; m/e 676 (M+); δ -3.24 (2H, s, NH), -2.9, -2.0, -1.0 (12H, m, bridge H), 1.81 (6H, t, J 7.5 Hz, Et CH_3), 3.2 (4H, m, $COOCH_2$), 3.4 (4H, m, CH_2COO), 3.61 and 3.64 (each 6H, s, ring Me), 4.07 (4H, q, J 7.5 Hz, Et CH_2), 4.3 and 4.7 (each 2H, m, CH_2CH_2COO), 10.34 (2H, s, 5,15-H), 10.40 (2H, s, 10,20-H).



(35)

17-Ethyl-3,8,13,18-tetramethyl-21H,23H-porphine-2,7,12-tripropanoic acid, 2,12-diethyl ester (35).- The dibromodipyrromethene (40) (1.05 g, 1.9 mM) and the α-free dipyrromethene (48) (0.83 g, 1.9 mM) were dissolved in dry dichloromethane (20 ml) and acetic acid (5 ml). Tin (IV) chloride (2 ml) was added under nitrogen and the mixture stirred in the dark in a nitrogen atmosphere for $2\frac{1}{2}$ h. 10% aqueous hydrobromic acid saturated with ammonium bromide (30 ml) was then added and stirred for 10 min. The inorganic solids which precipitated were removed by filtration and washed with dichloromethane. The combined organic layer was washed three times with a mixture of water (30 ml) and 10% aqueous hydrobromic acid saturated with ammonium bromide (30 ml). After evaporation, it gave a solid having $\lambda_{max.}$ 452 and 530 nm (no peak at 500 nm characteristic of the methenes), the biladiene. This was at once cyclised to the porphyrin by stirring in dimethyl sulphoxide (35 ml) and pyridine (4 ml) at 20° for 36 h. On evaporation of solvent, the residue was chromatographed twice on Silica (2 x 100 g Kieselgel), eluting with chloroform. Crystallisation from chloroform / ether gave the porphyrin diester (337 mg, 26.6%), m.p. 213-215° (Found: C, 69.83; H, 7.10; N, 7.89. $C_{39}H_{46}N_4O_6$ requires C, 70.25; H, 6.95; N, 8.40%), $\lambda_{max.}$ 400, 500, 533, 569, and 621 nm; $\nu_{max.}$ 3 320, 1 730, and 1 710 cm^{-1}; m/e 666 (M+); δ -3.1 (2H, s, NH), 1.01 (6H, t, J 7.1 Hz, ester Me), 1.85 (3H, t, J 7.5 Hz, Et CH_3), 3.45 (6H, t, J 7.5 Hz, CH_2COO), 3.58 (3H, s, 18-Me), 3.61 and 3.62 (each 3H, s, 3,13-Me), 3.68 (3H, s, 8-Me), 4.08 (2H, q, J 7.5 Hz, Et CH_2), 4.13 (4H, q, J 7.1 Hz, ester CH_2), 4.55 (4H, t, J 7.5 Hz, $C\underline{H}_2CH_2COOEt$), 4.69 (2H, t, J 7.5 Hz, $C\underline{H}_2CH_2COOH$), 10.34 (1H, s, 15-H), 10.45 (2H, s, 10,20-H), 10.57 (1H, s, 5-H).

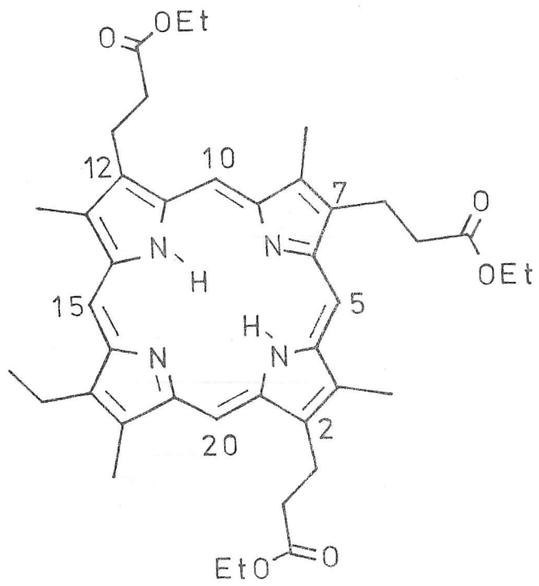
Alternatively, the dibromodipyrromethene (40) (4 g, 7.2 mM) was condensed with the α-COOH dipyrromethene (52) (3.5 g, 7.2 mM) by stirring in the dark in a nitrogen atmosphere with dichloromethane (80 ml), acetic acid (20 ml), and tin (IV) chloride (8 ml) for 10 days. The work-up procedure and



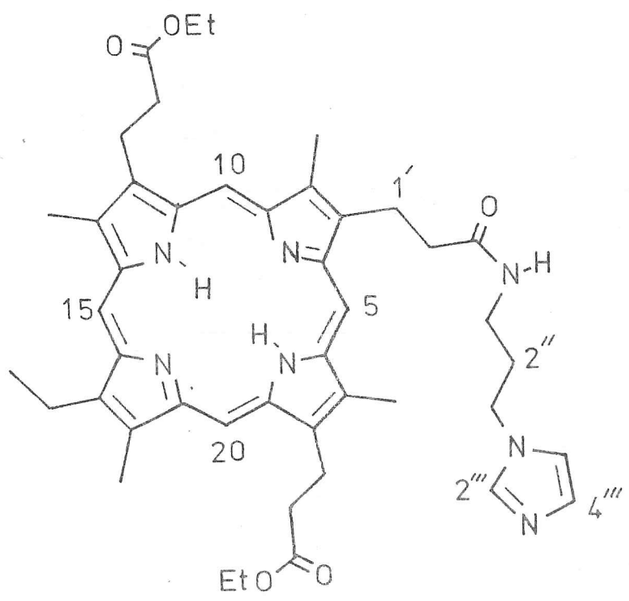
(36)

cyclisation to the porphyrin were essentially as described above for the combination of (40) and (48), and gave 654 mg (13.5%) of the porphyrin diester, identical to that produced by the other method.

17-Ethyl-3,8,13,18-tetramethyl-21H,23H-porphine-2,7,12-tripropanoic acid, 7-ethyl ester (36).- The dibromodipyrromethene (61) (2.17 g, 4.1 mM) and the dipyrromethene (62) (2 g, 4.1 mM) were treated under nitrogen in dry dichloromethane (80 ml) containing acetic acid (20 ml) with tin (IV) chloride (5 ml). The mixture was put aside for 14 days, when the visible spectrum showed complete conversion to the biladiene ($\lambda_{max.}$ 452 and 530 nm, no peak at 500 nm). 10% aqueous hydrobromic acid saturated with ammonium bromide (50 ml) was then added and stirred for 15 min. The inorganic solids which precipitated were removed by filtration and washed with dichloromethane. The combined organic layer was washed three times with a mixture of water (50 ml) and 10% aqueous hydrobromic acid saturated with ammonium bromide (50 ml). After evaporation, the solid produced was cyclised to the porphyrin by stirring in dimethyl sulphoxide (50 ml) and pyridine (10 ml) at 20° for 36 h. On evaporation of solvent, the residue was chromatographed on silica (120 g Kieselgel), eluting with chloroform. Crystallisation from chloroform / ether gave the porphyrin ester (0.85 g, 32%), m.p. 277-279° (Found: C, 69.43; H, 6.62; N, 8.56. $C_{37}H_{42}N_4O_6$ requires C, 69.57; H, 6.63; N, 8.77%), $\lambda_{max.}$ 396, 497, 530, 566, and 617 nm; $\nu_{max.}$ 3 320, 1 730, and 1 705 cm^{-1}; m/e 638 (M+); δ -3.1 (2H, s, NH), 1.01 (3H, t, J 7.1 Hz, ester Me), 1.84 (3H, t, J 7.5 Hz, Et CH_3), 3.53 (6H, t, J 7.5 Hz, CH_2COO), 3.56 (3H, s, 18-Me), 3.62 (3H, s, 8-Me), 3.67 (6H, s, 3,13-Me), 4.06 (2H, q, J 7.5 Hz, Et CH_2), 4.13 (2H, q, J 7.1 Hz, ester CH_2), 4.55 (2H, t, J 7.5 Hz, CH_2CH_2COOEt), 4.69 (4H, t, J 7.5 Hz, CH_2CH_2COOH), 10.34 (1H, s, 15-H), 10.46 (1H, s, 5-H), 10.57 (2H, s, 10,20-H).



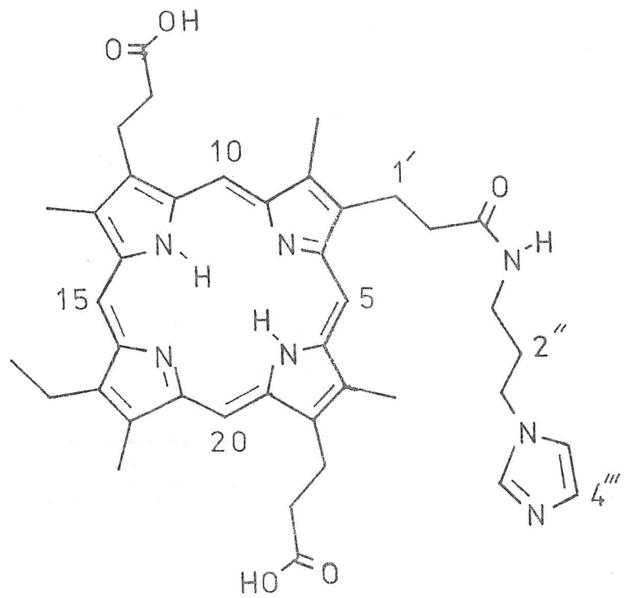
(36a)



(55)

Triethyl 17-Ethyl-3,8,13,18-tetramethyl-21H,23H-porphine-2,7,12,-tripropanoate (36a).- Either (35) or (36) could be esterified fully in ethanol containing sulphuric acid. Crystallisation of the porphyrin which resulted from chloroform / methanol gave the same triester, m.p. 160-162° (Found: C, 71.10; H, 7.21; N, 8.18. $C_{41}H_{50}N_4O_6$ requires C, 70.87; H, 7.25; N, 8.06%), $\lambda_{max.}$ 400, 500, 535, 570, and 624 nm; $\nu_{max.}$ 3 300 and 1 730 cm^{-1}; δ -3.15 (2H, s, NH), 0.99 (9H, t, J 7.1 Hz, ester Me), 1.83 (3H, t, J 7.5 Hz, Et CH_3), 3.44 (6H, t, J 7.5 Hz, CH_2COO), 3.56 (3H, s, 18-Me), 3.61 (9H, s, 3,8,13-Me), 4.07 (2H, q, J 7.5 Hz, Et CH_2), 4.11 (6H, q, J 7.1 Hz, ester CH_2), 4.55 (6H, t, J 7.5 Hz, CH_2CH_2COO), 10.33 (1H, s, 15-H), 10.45 (3H, s, 5,10,20-H).

17-Ethyl-7-[3-[[3-(1H-imidazol-1-yl)propyl]amino]-3-oxopropyl]-3,8,13,18-tetramethyl-21H,23H-porphine-2,12-dipropanoic acid, diethyl ester (55).- The above porphyrin diester (35) (290 mg, 0.44 mM) in dry dichloromethane (15 ml) was treated with oxalyl chloride (0.4 ml) and dimethyl formamide (2 μl) at 20° for 15 min. The solvent was removed by evaporation and the residue treated in dry dichloromethane (10 ml) with the amine free base (54) (0.75 g). After 1 h, the product was extracted into dichloromethane and the organic layer washed with water and brine, dried over sodium sulphate and evaporated. Crystallisation from chloroform / ether gave the tail diester (291 mg, 86%), m.p. 215-217°, $\lambda_{max.}$ 400, 500, 533, 569, and 621 nm; $\nu_{max.}$ 3 320, 1 730, and 1 645 cm^{-1}; m/e 773 (M+); δ -3.15 (2H, s, NH); 1.00 and 1.02 (each 3H, t, J 7.1 Hz, ester Me), 1.44 (2H, dt, J 6.7 Hz, 2''-CH_2), 1.83 (3H, t, J 7.5 Hz, Et CH_3), 3.15 (2H, m, $CONCH_2$), 3.3 (2H, t, J 6.7 Hz, CH_2Im), 3.45 (6H, t, J 7.5 Hz, CH_2COO and CH_2CON), 3.59 and 3.62 (12H, ring Me), 4.04 (2H, q, J 7.5 Hz, Et CH_2), 4.13 (4H, q, J 7.1 Hz, ester CH_2), 4.55 (4H, t, J 7.5 Hz, CH_2CH_2COO), 4.63 (2H, t, J 7.5 Hz, CH_2CH_2CON), 6.4 and 6.9 (each 1H, s, 4''',5'''-H), 7.29 (1H, s, 2'''-H), 8.45 (1H, m, CONH), 10.34 (1H, s, 10-H), 10.42 and 10.46 (each 1H, s, 5,15-H), 10.53 (1H, s, 20-H).



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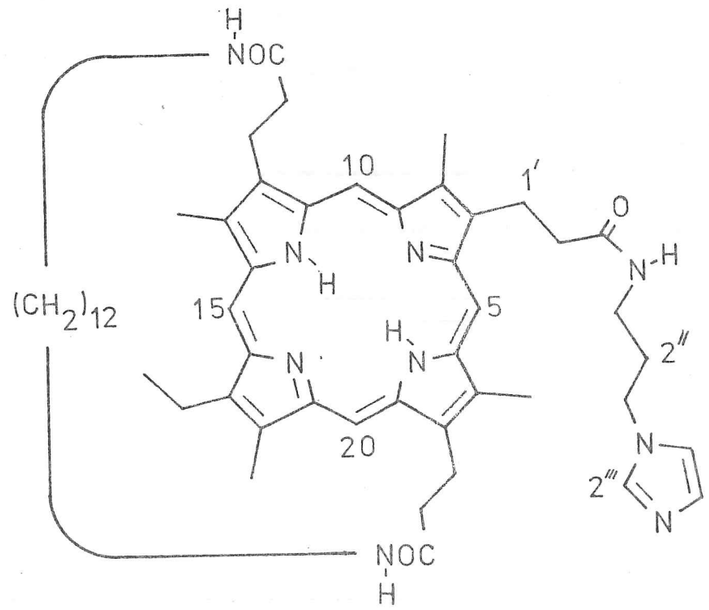
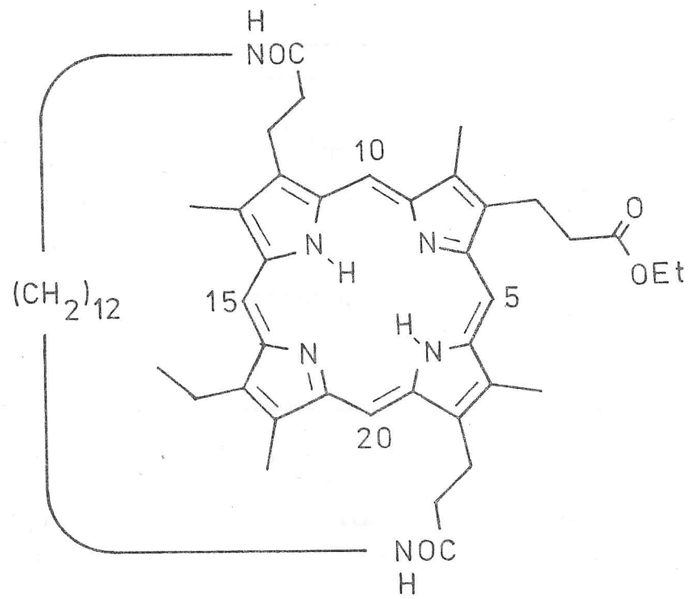


(57)

17-Ethyl-7-[3-[[3-(1H-imidazol-1-yl)propyl]amino]-3-oxopropyl]-3,8,13,18-tetramethyl-21H,23H-porphine-2,12-dipropanoic acid (56).- The above tail diester (55) (230 mg, 0.3 mM) was hydrolysed by stirring at 20° in 6N hydrochloric acid (30 ml) for 6 h. On evaporation of the solvent, the residue was dissolved in 2N hydrochloric acid (10 ml) and the pH adjusted to 5 with 2N aqueous potassium hydroxide, when the diacid precipitated. The solid was removed by centrifugation and was washed several times with water, to give the tail diacid (175 mg, 81.6%) after drying, m.p. 194-197°, $\nu_{max.}$ 1 705 and 1 630 cm^{-1}; δ 1.39 (2H, dt, J 6.7 Hz, 2''-H), 1.83 (6H, t, J 7.5 Hz, Et CH_3), 3.15 (2H, m, $CONCH_2$), 3.3 (2H, m, CH_2Im), 3.4 (6H, m, CH_2COO and CH_2CON), 3.56, 3.61, 3.66, and 3.68 (each 3H, s, ring Me), 4.08 (4H, q, J 7.5 Hz, Et CH_2), 6.33 and 6.82 (each 1H, s, 4''',5'''-H), 7.41 (1H, s, 2'''-H), 8.56 (3H, NH and COOH), 10.33 (1H, s, 15-H), 10.53, 10.54 (each 1H, s, 10,20-H), 10.57 (1H, s, 5-H).

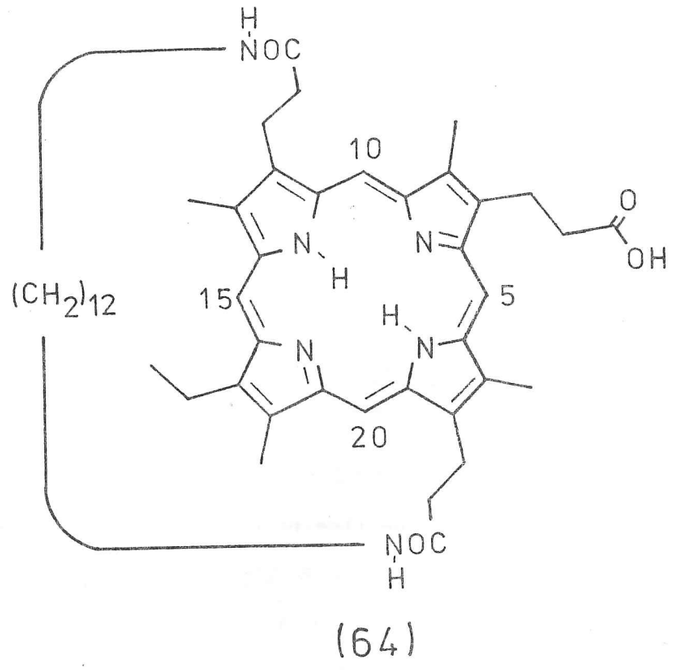
Bridge plus tail porphyrin (57).- The bridged porphyrin free acid (64) (307 mg, 0.4 mM) in dry dichloromethane (15 ml) was treated with oxalyl chloride (0.3 ml) at 20° for 20 min. After evaporation of the solvent and excess reagent, the residue in dry dichloromethane (20 ml) was treated with the amine free base (54) (0.75 g) at 20° for 30 min. The solvent was removed by evaporation and pyridine (1 ml) added: the product was then extracted into chloroform / methanol and washed with water. The organic layer was concentrated and the residue chromatographed on two silica thick-layer plates (0.5 mm), eluting with 15% methanol in chloroform. The product band was isolated and the recovered porphyrin crystallised from chloroform / ether to give the bridge plus tail compound (124 mg, 35%), m.p. 166-169°, $\lambda_{max.}$ 401, 501, 534, 570, and 622 nm; $\nu_{max.}$ 3 300, 1 640, and 1 540 cm^{-1}; m/e 881 (M+); δ -3.14 (2H, s, Ar-NH), -0.78, 0.05, and 0.83 (20H, m, bridge H), 1.55 (2H, dt, 2''-H), 1.9 (3H, t, J 7.5 Hz, Et CH_3), 3.2 (4H, m, 1'',3''-H), 3.37 (4H, m, $CONCH_2$-bridge), 3.4 (6H, m, CH_2CON), 3.63, 3.64, 3.68, and 3.69 (each 3H,



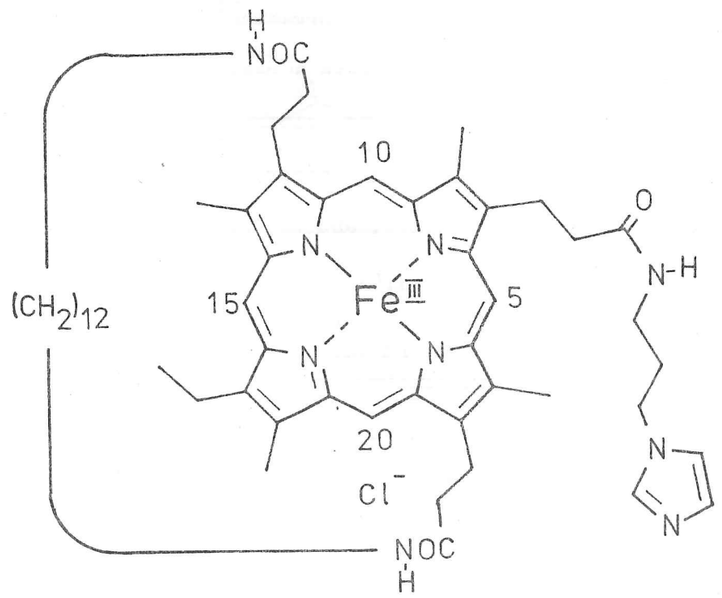
(63)

s, ring Me), 4.10 (2H, q, J 7.5 Hz, Et CH_2), 4.12 and 4.7 (each 2H, m, CH_2CH_2CON-bridge), 4.7 (2H, m, CH_2CH_2CON-tail), 6.54 and 6.94 (each 1H, s, 4''',5'''-H), 7.54 (1H, s, 2'''-H), 8.29 (2H, t, CONH-bridge), 8.5 (1H, t, CONH-tail), 10.31 (1H, s, 15-H), 10.54 and 10.56 (each 1H, s, 10,20-H), 10.66 (1H, s, 5-H).

Bridged porphyrin, propanoate ethyl ester (63).- The above porphyrin diacid (36) (1 g, 1.57 mM) in dry dichloromethane (15 ml) was treated with oxalyl chloride (1 ml) and dimethyl formamide (2 μl) at 20° for 15 min. The solution was evaporated and the residue (the porphyrin diacid chloride) in dry dichloromethane (200 ml) added dropwise to dry dichloromethane (250 ml) while dodecane-1,12-diamine (470 mg, 2.35 mM) in dry dichloromethane (250 ml) containing pyridine (1 ml) was simultaneously added. This took 2 h, and after stirring for a further 30 min, another portion of the solid diamine (100 mg) was added. Then, after 1 h, the solvent was removed by evaporation until the total volume was 300 ml, and the organic layer washed with water. Subsequently, evaporation of that layer gave a residue which was chromatographed on neutral alumina (70 g, grade 3), eluting with chloroform. The product was crystallised from chloroform / ether to yield 480 mg (38.2%), m.p. 269-271°, $\lambda_{max.}$ 401, 501, 535, 570, and 623 nm; $\nu_{max.}$ 3 300, 1 730, and 1 640 cm^{-1}; m/e 802 (M+); δ -3.14 (2H, s, Ar-NH), -0.87, -0.03, and 0.8 (20H, m bridge H), 1.09 (3H, t, J 7.1 Hz, ester Me), 1.89 (3H, t, J 7.5 Hz, Et CH_3), 3.38 (4H, m, $CONCH_2$), 3.4 (4H, m, CH_2CON), 3.49 (2H, t, CH_2COO), 3.61 and 3.62 (each 3H, s, 8,18-Me), 3.67 (3H, s, 13-Me), 3.72 (3H, s, 3-Me), 4.09 (2H, q, J 7.5 Hz, Et CH_2), 4.1 and 4.8 (each 2H, m, CH_2CH_2CON), 4.58 (2H, t, CH_2CH_2COO), 8.25 (2H, t, CONH), 10.31 (1H, s, 15-H), 10.42 (1H, s, 5-H), 10.59 and 10.63 (each 1H, s, 10,20-H).



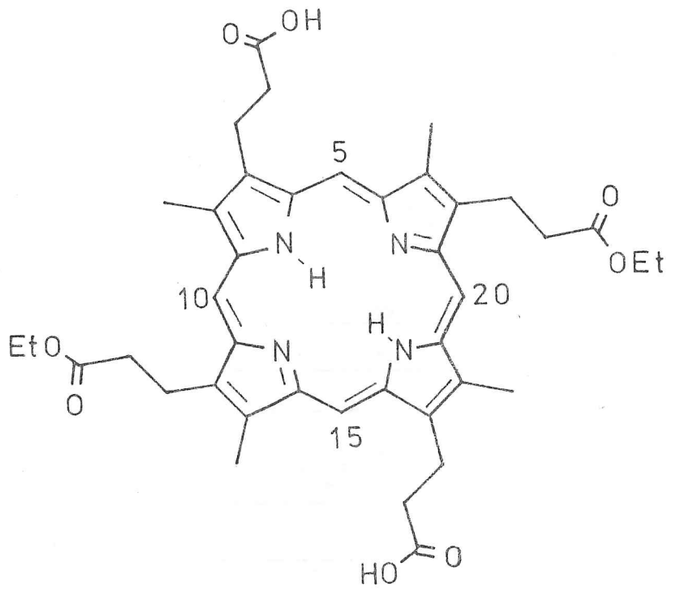
(64)



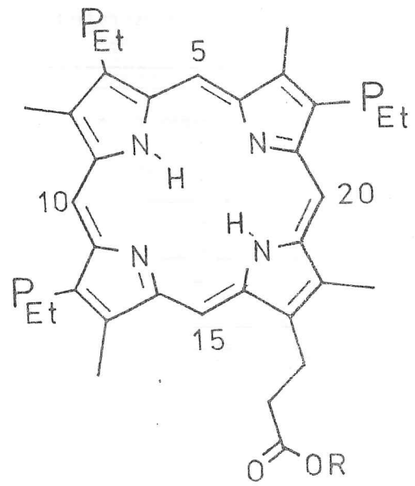
(65)

Bridged porphyrin, propanoic acid (64).- The foregoing ethyl ester (63) (410 mg, 0.51 mM) was hydrolysed at 20° in 9N hydrochloric acid (60 ml) for 3 h. On evaporation of the solvent, pyridine (2 ml) was added and, after 5 min, removed by evaporation. The residue was taken in chloroform and washed with water. Evaporation of the organic layer and crystallisation from chloroform / ether gave the bridged porphyrin free acid (327 mg, 82.6%), m.p. 280-283°, $\lambda_{max.}$ 401, 501, 535, 570, and 623 nm; $\nu_{max.}$ 3 300, 1 715, and 1 640 cm^{-1}; m/e 774 (M+); δ -3.14 (2H, s, Ar-NH), -0.82, 0.02, and 0.78 (20H, m, bridge H), 1.9 (3H, t, J 7.5 Hz, Et CH_3), 3.24 (4H, m, $CONCH_2$), 3.4 (6H, m, CH_2COO and CH_2CON), 3.59 (3H, s, 18-Me), 3.63 (3H, s, 13-Me), 3.68 (3H, s, 3-Me), 3.75 (3H, s, 8-Me), 4.1 (2H, q, J 7.5 Hz, Et CH_2), 4.3 and 4.8 (each 2H, m, CH_2CH_2CON), 4.71 (2H, t, CH_2CH_2COO), 8.22 (2H, t, CONH), 10.31 (1H, s, 15-H), 10.52 and 10.56 (each 1H, s, 10,20-H), 10.62 (1H, s, 5-H).

Bridge plus tail porphyrin, iron (III) complex, chloride (65).- The porphyrin (57) (40 mg, 4.5×10^{-5} M) was metallated with ferrous sulphate (40 mg), essentially as described for the preparation of (24). The metallation was carried out in a Craig tube to facilitate washing of the product with removal of the solvent by centrifugation and decantation. The iron complex (35 mg, 80%) had m.p. >300°, $\lambda_{max.}$ 404, 529, and 635 nm.



(67)



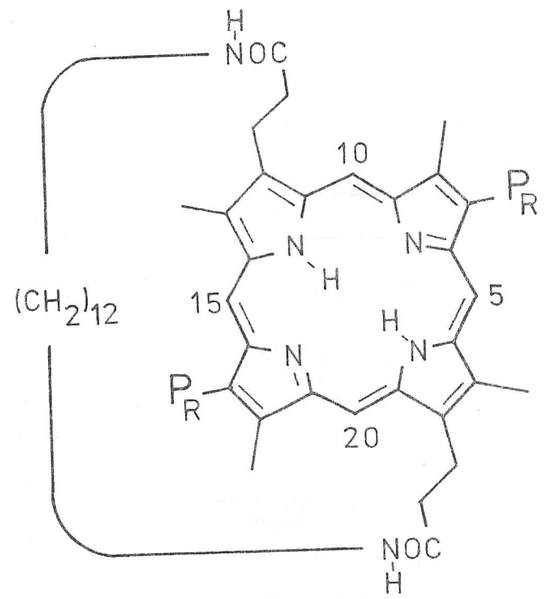
(68) R = H

(69) R = Et

3,8,13,18-Tetramethyl-21H,23H-porphine-2,7,12,17-tetrapropanoic acid, 2,12-diethyl ester (67).- The dipyrromethene (62) (2.83 g, 5.86 mM) and the dibromodipyrromethene (66) (3.5 g, 5.86 mM) were treated essentially as described above for the formation of the porphyrin (36). A minor by-product of the chromatography was the triester (68). The major product was the desired diester (1.1 g, 26.4%), m.p. 255-258° (from chloroform / ether) (Found: C, 66.33; H, 6.8; N, 7.32. $C_{40}H_{46}N_4O_8.H_2O$ requires C, 65.91; H, 6.64; N, 7.69%), $\lambda_{max.}$ 400, 500, 534, 570, and 621 nm; $\nu_{max.}$ 3 310, 1 730 (sh), and 1 705 cm^{-1}; m/e 710 (M+); δ -3.1 (2H, s, NH), 1.01 (6H, t, J 7.1 Hz, ester Me), 3.43 (8H, t, J 7.6 Hz, CH_2COO), 3.61 (6H, s, 3,13-Me), 3.67 (6H, s, 8,18-Me), 4.13 (4H, q, J 7.1 Hz, ester CH_2), 4.54 (4H, t, J 7.6 Hz, $C\underline{H}_2CH_2COOEt$), 4.69 (4H, t, J 7.6 Hz, $C\underline{H}_2CH_2COOH$), 10.44 (2H, s, 10,20-H), 10.56 (2H, s, 5,15-H).

3,8,13,18-Tetramethyl-21H,23H-porphine-2,7,12,17-tetrapropanoic acid, 2,7,12-triethyl ester (68).- A minor (ca. 20 mg) by-product of the chromatography in the preparation of the above porphyrin (67) was the triester, which was crystallised from dichloromethane / ethanol, m.p. 170-172°, m/e 738; δ -3.1 (2H, s, NH), 1.00 (9H, t, J 7.1 Hz, ester Me), 3.44 (8H, t, J 7.5 Hz, CH_2COO), 3.62 (9H, s, 3,8,13-Me), 3.65 (3H, s, 18-Me), 4.12 (6H, q, J 7.1 Hz, ester CH_2), 4.5 (6H, t, J 7.5 Hz, $C\underline{H}_2CH_2COOEt$), 4.7 (2H, t, J 7.5 Hz, $C\underline{H}_2CH_2COOH$), 10.43 (3H, s, 5,10,20-H), 10.54 (1H, s, 15-H).

Tetraethyl 3,8,13,18-Tetramethyl-21H,23H-porphine-2,7,12,17-tetrapropanoate (Coproporphyrin I tetraethyl ester) (69).- The porphyrin (67) could be fully esterified in ethanolic acid to give, after crystallisation from chloroform / ethanol the tetraethyl ester, m.p. 222-223.5° (lit., [52] 225-226°) (Found: C, 68.85; H, 7.13; N, 7.18. $C_{44}H_{54}N_4O_8$ requires C, 68.90; H, 7.10; N, 7.31%), δ -3.19 (2H, s, NH), 0.99 (12H, t, J 7.1 Hz, ester Me), 3.43 (8H, t, J 7.5 Hz, CH_2COO), 3.61 (12H, s, ring Me), 4.11 (8H, q, J 7.1 Hz, ester CH_2), 4.54 (8H, t, J 7.5 Hz, $C\underline{H}_2CH_2COO$), 10.43 (4H, s, meso H).



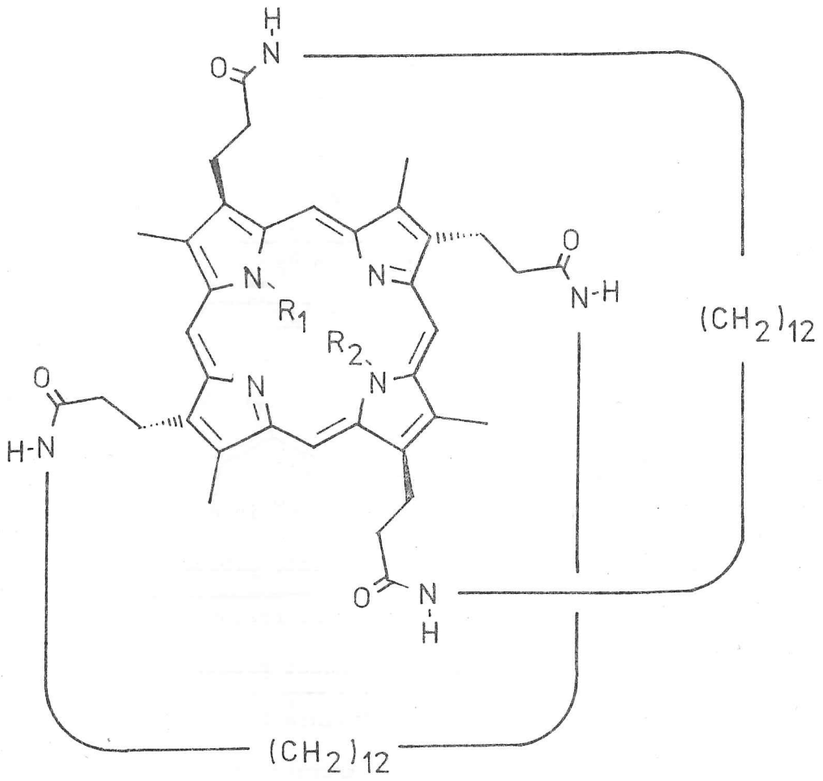
(71) R = Et

(72) R = H

Bridged porphyrin, bis ethyl propanoate (71).- The above porphyrin (67) (1.08 g, 1.52 mM) was treated essentially as described for the preparation of the bridged compound (63). After chromatography and crystallisation from chloroform / ether, the bridged diester (445 mg, 33.5%) was obtained, m.p. 161-163°, $\lambda_{max.}$ 400, 500, 534, 570, and 623 nm; $\nu_{max.}$ 3 300, 1 730, and 1 640 cm^{-1}; m/e 874 (M+); δ -3.15 (2H, s, NH), -0.87, 0.03, and 0.82 (20H, m, bridge H), 1.1 (6H, t, J 7.5 Hz, ester Me), 3.3 (4H, m, $CONCH_2$), 3.4 (4H, m, CH_2CON), 3.49 (4H, t, CH_2COO), 3.61 (6H, s, 8,18-Me), 3.73 (6H, s, 3,13-Me), 4.1 and 4.8 (each 2H, m, CH_2CH_2CON), 4.18 (4H, q, J 7.5 Hz, ester CH_2), 4.57 (4H, t, CH_2CH_2COO), 8.24 (2H, t, CONH), 10.41 (2H, s, 5,15-H), 10.62 (2H, s, 10,20-H).

Bridged porphyrin, bis propanoic acid (72).- The foregoing ester (71) (757 mg, 0.87 mM) was hydrolysed at 20° in 8N hydrochloric acid (100 ml) for 4 h. On evaporation of the solvent, pyridine (3 ml) was added and, after 10 min, removed by evaporation. The residue was taken in chloroform / ethanol and washed with water. The product precipitated and was recovered by filtration. A second batch was obtained by evaporation of the organic layer. These were combined and crystallised from chloroform / methanol / ether to give the bridged diacid (620 mg, 87.5%), m.p.>300°, $\lambda_{max.}$ (EtOH) 398, 500, 533, 572, and 622 nm; $\nu_{max.}$ 1 705 and 1 615 cm^{-1}; δ -3.06 (2H, s, Ar-NH), -0.79, 0.02, and 0.85 (20H, m, bridge H), 3.3 (4H, m, $CONCH_2$), 3.4 (6H, m, CH_2COO and CH_2CON), 3.62 (6H, s, 8,18-Me), 3.79 (6H, s, 3,13-Me), 4.1 and 4.8 (each 2H, m, CH_2CH_2CON), 4.72 (4H, t, CH_2CH_2COO), 8.23 (2H, t, CONH), 10.50 (2H, s, 5,15-H), 10.59 (2H, s, 10,20-H).

Double bridged porphyrin (73).- The above diacid (72) (600 mg, 0.73 mM) was treated essentially as described for the preparation of the bridged compound (63). After crystallisation from chloroform / ether, the double bridged porphyrin was obtained (83 mg, 11.5%), m.p.>300°,



(73) $R_1 = R_2 = H$

(74) $R_1 — R_2 = —\overset{III}{Fe}(Cl)—$

$\lambda_{max.}$ 402, 502, 536, 571, and 622 nm; $\nu_{max.}$ 3 300, 1 640, and 1 540 cm^{-1}; m/e 982 (M+); $\delta(CDCl_3)$ -0.2, 0.04, and 0.47 (40H, m, bridge H), 2.77 (8H, t, $CONCH_2$), 3.05 (8H, m, CH_2CON), 3.60 (12H, s, ring Me), 4.1 and 4.6 (each 4H, m, CH_2CH_2CON), 10.15 (4H, s, meso H).

δ (d_5-Pyridine) -3.1 (2H, s, Ar-NH), -0.6, 0.03, and 0.78 (40H, m, bridge H), 3.2 (8H, m, CH_2CON), 3.4 (8H, m, $CONCH_2$), 3.74 (12H, s, ring Me), 4.25 and 4.85 (each 4H, m, CH_2CH_2CON), 8.2 (4H, t, CONH), 10.59 (4H, s, meso H).

Double bridged porphyrin, iron (III) complex, chloride (74).- The above porphyrin (73) (53 mg, 5.4×10^{-5} M) was taken in a Craig tube with ferrous sulphate (50 mg), pyridine (100 μl), and acetic acid (1 ml) and heated on a steam-bath for 10 min. A mixture of brine (0.5 ml) and water (0.5 ml) was then added and heated for 2 min before allowing to cool. The tube was centrifuged and the supernatant removed. The residue was dried under high vacuum and then chromatographed on neutral alumina (10 g, grade 3), eluting with 5% methanol in chloroform. The porphyrin which was recovered was crystallised from acetic acid / 1:1 brine:water in a centrifuge tube and was then washed with water by decantation. After drying under high vacuum, the iron porphyrin was obtained (55.5 mg, 94%), m.p. >300° (Found: C, 66.18; H, 8.00; N, 10.01. $C_{60}H_{84}N_8O_4FeCl.H_2O$ requires C, 66.07; H, 7.95; N, 10.27%), $\lambda_{max.}$ 379, 507, 537, and 638 nm.

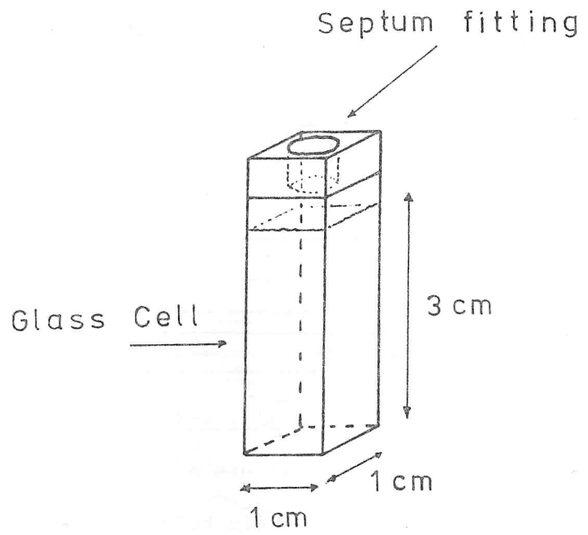


Figure 35

e) Oxygen-binding studies

The oxygen-binding properties of model iron porphyrins, which are described in Chapter 2 section (d) and Chapter 3 section (d), were determined by visible spectroscopy. As the ferrous compounds were all highly sensitive to oxygen, procedures were developed for manipulating solutions under rigorously oxygen-free atmospheres. The general methodology was based on the techniques described in the excellent reference books by D. F. Shriver [128] and H. C. Brown [129].

There were two requirements for successfully obtaining visible spectra. One was to generate the ferrous species in an oxygen-free solution, and the other to transfer it to a spectrometer cell for spectral measurements. The cell used was of a conventional type — having a 1 cm path length, and holding ca. 3 ml of solution. It had a circular aperture which would take a suitably-sized rubber septum through which the solution to be examined might be transferred from a syringe (Figure 35). An optically matched reference cell was available.

In the simplest experiments, it was possible to inject a solution of the reducing agent (e.g. anhydrous hydrazine or chromium (II) (acetyl-acetonate)$_2$ [130]) directly into the cell containing the porphyrin. However, in many cases, it was desirable to prepare larger (ca. 20 ml) amounts of the reduced material in solution and to transfer aliquots to the cell for oxygen and carbon monoxide binding experiments. The chosen reagent for these reductions was aqueous sodium dithionite, and the porphyrin was dissolved in a water-miscible solvent such as acetone or tetrahydrofuran. Many reducing agents have been used in porphyrin studies [75], and sodium dithionite is one of the most convenient.

Schlenk-type equipment was used to hold the solutions. Flasks were modified to accomodate side-arms having teflon taps, so that they might be evacuated and the experiments performed under oxygen-free nitrogen.

Experiments carried out in glove boxes, which are an alternative source of oxygen-free atmospheres [128] were less successful with the equipment that was available, and were rejected in favour of the Schlenk-tube procedures.

The nitrogen supply was commercial "white-spot" high-purity nitrogen, specified to have an oxygen content of less than 5 ppm. This was passed through an aqueous solution of chromium (II) chloride to remove last traces of oxygen, as recommended in D. F. Shriver's book [128]. The resultant nitrogen was estimated to have less than 2 ppm residual oxygen. It was passed through a tower of potassium hydroxide to remove acid vapours and moisture, and was then dispensed from the apparatus shown in Figure 36. This allowed the Schlenk equipment to be flushed with nitrogen without at any stage opening it to the air.

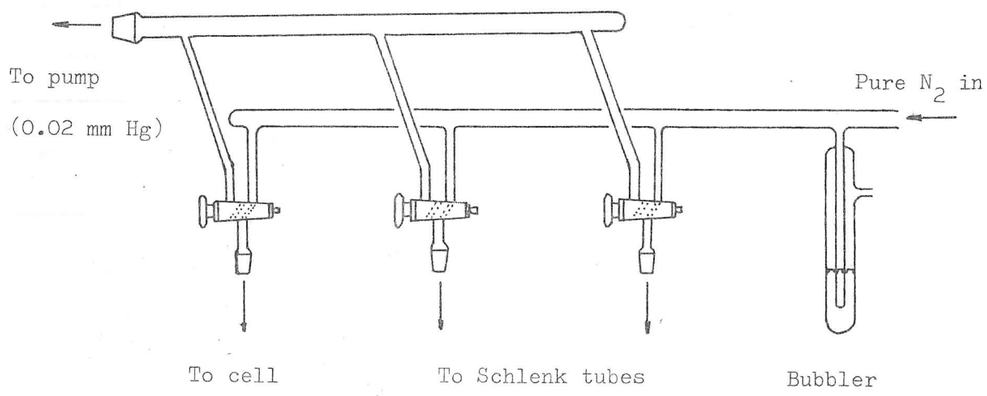


Figure 36

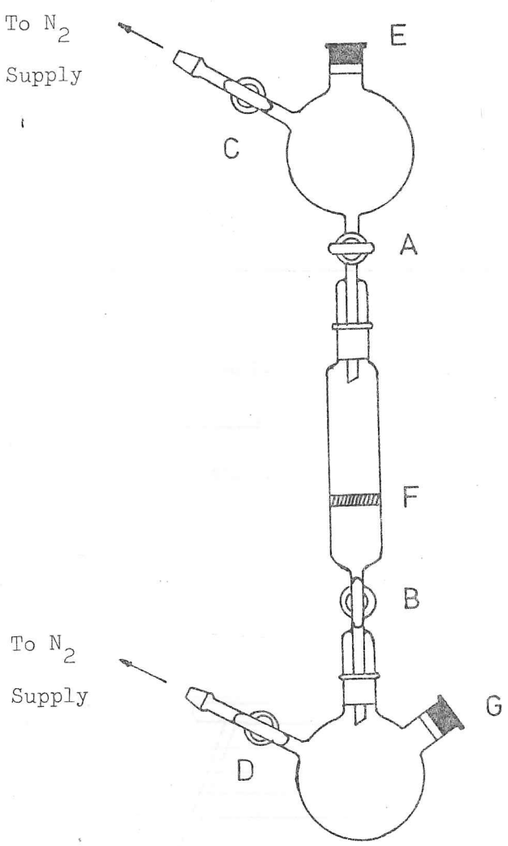


Figure 37

The apparatus used for the reductions is shown in Figure 37. Porphyrin solutions were made up in degassed solvents to a suitable concentration, which was monitored by the visible spectrum. They were placed in the dropping funnel with tap A closed and tap B open. The atmospheres in the two compartments were converted to pure nitrogen by alternately evacuating through taps C and D and readmitting nitrogen from the supply (four flushing cycles were performed).

Aqueous sodium dithionite was injected through septum E to reduce the metalloporphyrin. This reaction gave a precipitate of sodium salts, which was removed when tap A was subsequently opened and the solution transferred under reduced pressure through the sintered frit F to the lower flask. After closing tap B at the end of this operation, aliquots of the solution were available through septum G. N-methyl imidazole could also be admitted through G as required. Carbon monoxide or oxygen could be blown through the porphyrin solutions, either in the lower flask or after an aliquot had been transferred to the spectrometer cell.

This transfer was done by the "three-needle" technique [128], to maintain a nitrogen atmosphere within the cell, as shown in Figure 38. Spectra were then readily obtained on a conventional spectrophotometer (see p. 23 and 47).

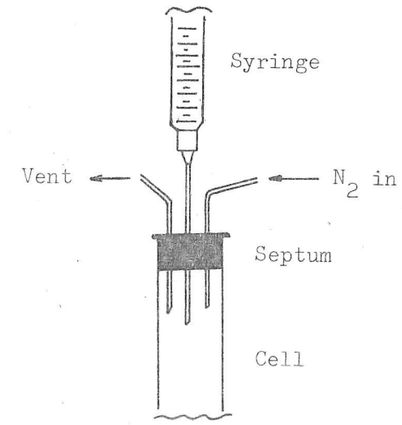


Figure 38

CHAPTER SIX CONCLUSIONS

a) Summary

The work described in the previous chapters has shown how a range of functionalised etioporphyrins can be used to prepare a series of models for the active sites of the haemoproteins. In terms of the objectives of the project, which are enumerated on p. 10 above, it can be said that three out of the four have been partially met.

Flexible and high-yielding routes to porphyrins which have an electronic structure which is very similar to that of the natural haems have been developed. Applications in the modelling of the cytochromes and chlorophyll are within sight. There is every reason to suppose that appended ligands can be attached to the porphyrins which have been prepared, to duplicate the ligands present in any given haemoprotein.

In addition, the n.m.r. work described in Chapter 4 shows the power of that technique in reaching a detailed conclusion concerning the structure of the molecules that have been prepared, and future work with metal derivatives of these porphyrins may be expected to give insights into the electronic configuration and redox properties.

However, a notable shortcoming has been the failure of any of the model systems to duplicate the key function of the myoglobin active site they were designed to mimic — its reversible oxygen-carrying rôle. This is a surprising development in view of the close structural similarity to the prosthetic group (arguably closer than in any other model, with the exception of some polymer-supported ones [30]). The low molecular weight models which do reversibly bind oxygen at 20° are meso-tetraphenyl types, and it is tempting to speculate that the success of these entities is precisely because they do <u>not</u> entirely duplicate all the structural and electronic features of ferroprotoporphyrin IX and its surrounding peptide.

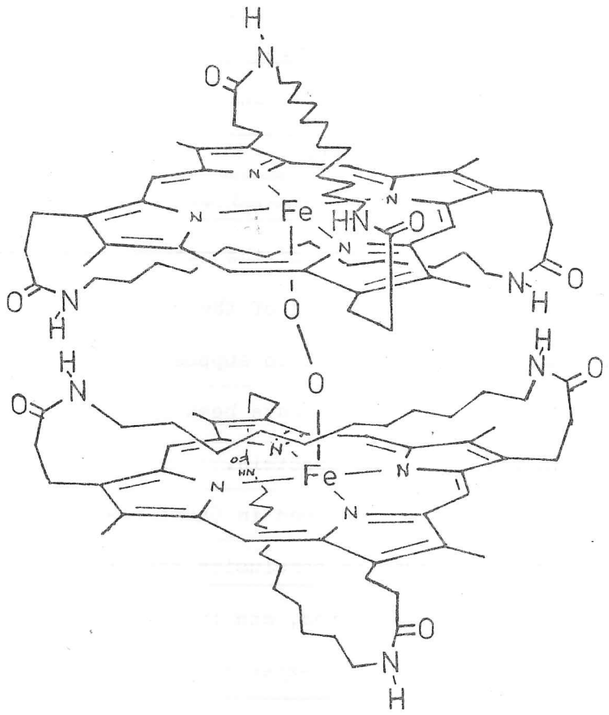


Figure 39

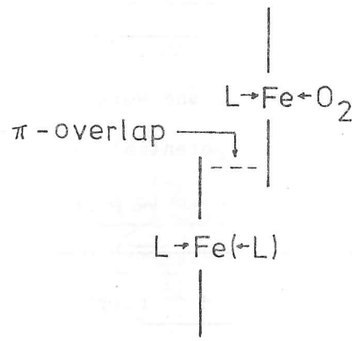
In this chapter, a brief attempt will be made to resolve this paradox and to suggest future experiments; bringing together all the available information in this rapidly developing field. It should be stated at the outset that two assumptions are inherent in what will follow. These are:

1) that the outcome of the oxygen-binding experiments described in Chapters 2 (d) and 3 (d) truly represent an inability of these models to maintain oxygenated ferrous forms and that oxidation is the inevitable result of admitting oxygen at 20°, and

2) that the doubly bridged metalloporphyrin (74) cannot oxidise by a mechanism which demands the face-to-face juxtaposition of two molecules to create an Fe-O-O-Fe intermediate.

The first assumption is supported by the work of J. E. Baldwin's and H. Ogoshi's groups [88, 89]. Whenever a potential oxygen-carrying model contains a free meso position, it is unable to support oxygenation without oxidation if the porphyrins are free to approach one another in solution: polymer supported [30] and micelle isolated [33] porphyrins are examples where reversible oxygenation can occur. The only published possible counter-example is C. K. Chang's polyether "crowned" porphyrin [81]. This result must be accomodated in any complete picture of the chemistry (see p. 125).

The second assumption is less well supported. It is unlikely, in view of the structure of the complex, that two molecules could come within bonding distance at their centre, for, as shown in Figure 39, this would necessitate that the bridges be forced out of the way on either side of the cavity. However, it is not impossible, and may yet be shown to be exactly what does occur!



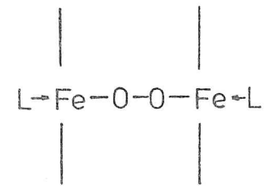
(XIV)

Figure 40

b) A possible new mechanism for irreversible oxidation

It will now be argued that all the results which are presently available may be rationalised by postulating that a mechanism for the oxidation of meso-unsubstituted iron porphyrins exists that is unavailable to those that are substituted at the meso positions. A likely candidate for this mechanism is an outer-sphere electron transfer process.

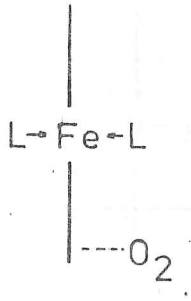
Evidence is now very strong that one pathway for oxidation of the oxygenated porphyrins involves an intermediate of the type (II):



(II)

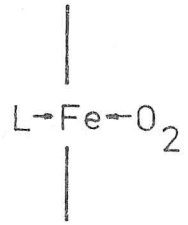
and an inner-sphere mechanism (one in which electron flow occurs through a ligand bound within the coordination sphere of both participating iron atoms). Recent n.m.r. work by G. N. La Mar et al. [131] presents direct evidence for this hitherto hypothetical intermediate (compare Figure 3 and discussion on p. 4), in their case without axial bases "L".

In contrast, the possibility of an outer-sphere electron transfer mechanism involving the transition state (XIV) (Figure 40) does not seem to have been considered in recent discussions of the mechanism of oxidation [132]. In early studies, O. H. W. Kao and J. H. Wang did allow for the possibility of partial reaction by an outer-sphere electron transfer (one in which electron flow occurs through ligands not mutually shared by coordination at the metal centres) [133]. They proposed that the porphyrin was remotely oxidised while maintaining a full complement of nitrogenous ligands, and hence that an intermediate like (XV) was implicated.



(XV)

Later studies by W. S. Caughey and I. A. Cohen [22] showed that the mechanism was more likely to be totally inner-sphere with regard to the oxygen, and that (XVI) was an obligatory intermediate.



(XVI)

The possibility that (XIV) might be involved was not considered by either group. It has elements of both ideas, and general enzyme intermediates of that type have been discussed by L. E. Bennett [134]. Outer-sphere electron transfer has been extensively researched in connection with the cytochromes, where rapid electron transfer between electron carriers in the respiratory chain occurs without apparent reorganisation of the ligands [7, 45, 135, 136]. These studies confirm the importance of this mechanism in metalloporphyrin reactions, and it has also been proposed as a mechanism for the slow oxidation of haemoglobin to methaemoglobin.

The hypothesis that the electron flow may occur through the porphyrin periphery provides an explanation for the difference between meso-substituted models and meso-free ones. The phenyl groups at right-angles to the porphyrin plane will put steric constraints on the closest approach that may occur in dimerisation. Overlap of the π-orbitals of the porphyrin will be necessary

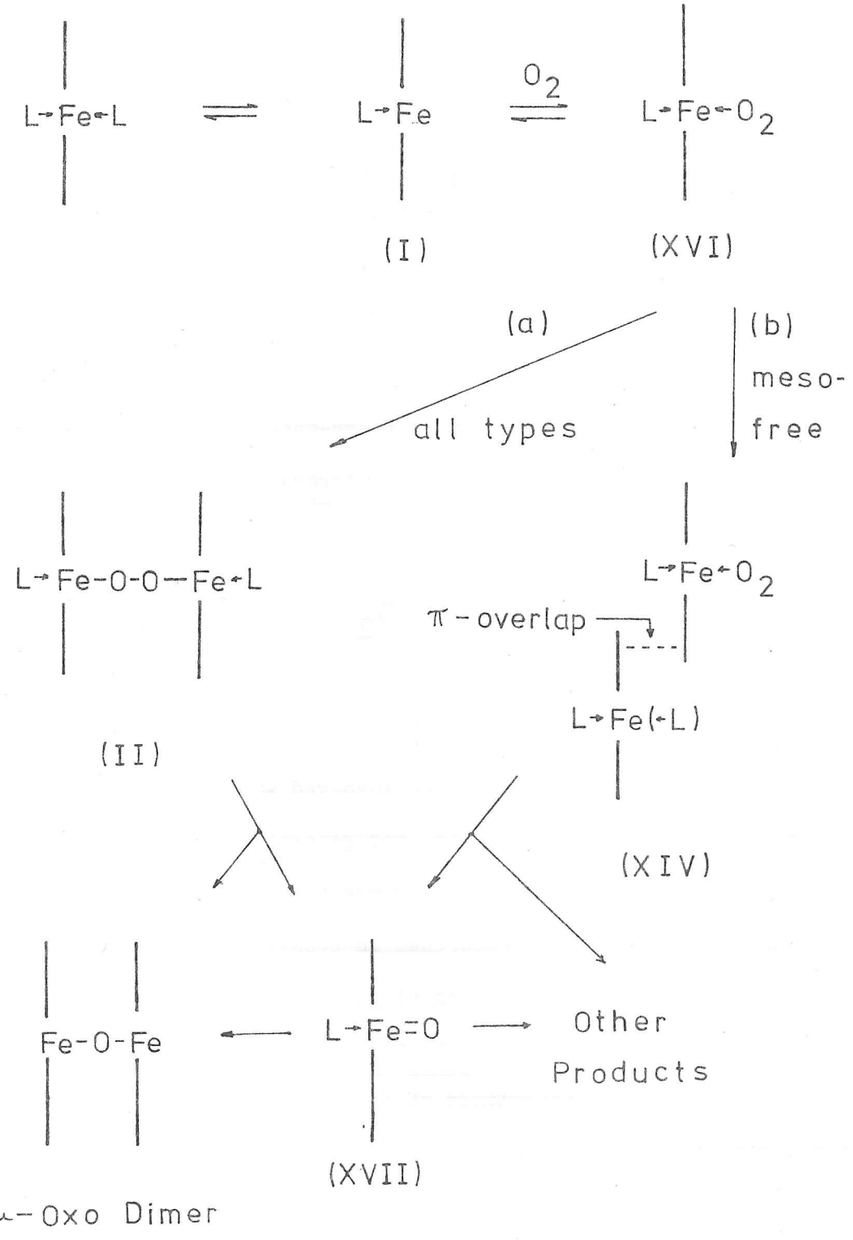
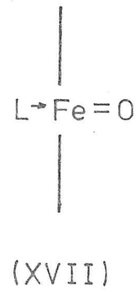


Figure 41

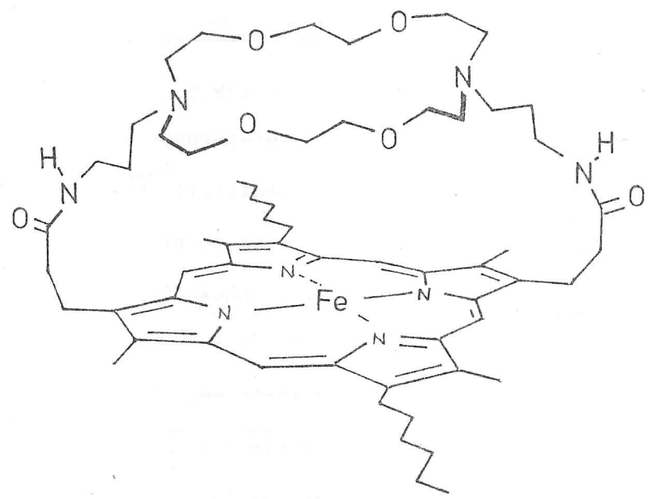
for electron transfer to occur, and this will require that they come within 3 to 4 Å of one another. This distance is typical for aromatic $\pi-\pi$ interactions [138], although the n.m.r. spectra of free-base porphyrins has been interpreted in terms of a closest approach of 7 Å [101]. Tight dimers have, however, been implicated in some etioporphyrin studies. G. N. La Mar *et al.* postulated an interplane separation of less than 4.5 Å in dicyanohaemin [139], and D. Mauzerall *et al.* presented evidence for zinc octaethylporphyrin cation radical dimers in Van der Waals contact [140].

Porphyrin face-to-face dimers are attracting attention for use in multi-electron reduction of oxygen (with possible applications in fuel cells) [141], but meso-substituted types do not appear to allow a sufficiently close approach of the two porphyrin planes for inter-porphyrin interactions [142].

There is sufficient precedent for the possibility of close overlap between the π-orbitals in meso-free porphyrins to propose that this may provide a competing pathway for oxidation, as shown in outline in Figure 41. The immediate result of electron transfer in an intermediate like (XIV) (in which *à priori* the non-oxygenated porphyrin may be five or six coordinated) may be a ferric porphyrin and either a porphyrin radical anion or an iron (IV) porphyrin. Protonation at the outer oxygen would allow its expulsion as a hydroxide ion, and might leave the intermediate (XVII) which has been proposed to take part in the oxidation process [132]. While protons are known to catalyse the oxidative reaction, they are not obligatory [132].



(XVII)



"Crowned Porphyrin"

Figure 42

c) Suggested further experiments

The above proposal to rationalise the oxidation of meso-free models is clearly highly speculative. Several experimental tests will now be suggested.

1) The double bridged metalloporphyrin (74) may be an example of a porphyrin for which only the path (b) in Figure 41 is available. If so, then the product of the oxidation will not be a μ-oxo dimer, but probably a monomeric porphyrin hydroxide. This would also be formed by treatment of the ferric compound directly with hydroxide ion, and would be an interesting candidate for X-ray analysis.

2) C. K. Chang's observation that the half-life for oxidation of the extremely bulky, but meso-free crowned porphyrin (Figure 42) [81] is only 3 minutes when N-methyl imidazole is used as axial base and rises to over an hour when the bulky base N-trityl imidazole is used, suggests that steric factors are of prime importance in preventing the "new" pathway for oxidation. This ligand may serve to convert the double bridged model (74) into a functioning oxygen-carrier, for when (or if) coordinated it should fully protect the faces from interacting even by partial overlap of the π system.

3) Alternatively, noting that Chang's porphyrin carries hexyl residues that must assist in fending off other porphyrins at the meso positions (which are the likely prime sites for the necessary orbital overlap), it would be interesting to replace the methyl and ethyl groups which were used in the β-positions of the pyrroles in the current work with bulkier substituents: isopropyl groups might be of sufficient size. This would seem to be a more attractive synthetic target than the alternative of adding phenyl groups to each meso position.

4) In view of G. N. La Mar's recent success in studying the intermediates in porphyrin oxidations by n.m.r. at low temperature [131], such experiments

should be repeated with both natural porphyrins (he used meso-substituted ones) and with some of the models like the double bridged porphyrin. At low temperature, the iron oxygen adduct is stable and diamagnetic, and varying the temperature slowly upwards from a very low value should allow the study of reaction intermediates in any porphyrin.

5) Etioporphyrins held together in face-to-face dimers are clearly of interest to determine the extent of interporphyrin interaction. H. Ogoshi's group have already reported such dimers, but these are held apart by over 4 Å with the linkages used [143]. The synthesis of dimers in closer contact should be investigated, and they will be available using simple porphyrins and techniques of the type described in this thesis.

In conclusion, the research possibilities in this field are still immense. Much more can be anticipated than has yet been achived!

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